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LOW FIBRINOLYTIC ACTIVITY IN THE WALLS OF VEINS IN PATIENTS WITH THROMBOSIS

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Abstract The fibrinolytic activity of biopsy specimens of veins from 58 patients with superficial thrombophlebitis and/or deep venous thrombosis of the limbs from nine healthy volunteers and from ten patients about to undergo biliary surgery has been studied by a modification of the fibrinolysis autography technique of Todd. The findings appeared to warrant the following conclusions:

1 The fibrinolytic activity of unaffected veins in the legs of patients with thrombosis and thrombophlebitis is significantly lower than that of veins in normals.

2 In veins occluded by compact thrombi the fibrinolytic activity is negligible but reappears when the thrombi become canalized.

It appears that low fibrinolytic activity of the vessel walls predisposes to thrombosis.

Using the so called "fibrinolysis autography technique" Todd (6) showed that the walls of blood vessels contain a fibrinolytic enzyme, the activator of plasminogen. It is believed that this enzyme is important in dissolving intravascular clots. (1) In postmortem studies Todd (7) rarely found fibrinolytic activity in areas where the thrombi were attached to the vessel walls but often in those where the thrombi had become detached. In experimental venous thrombosis in rats Kwaan and Astrup (3) demonstrated that cells from the vascular endothelium are involved in the resolution and canalization of thrombi. We recently observed (4) that the concentration of the plasminogen activator in the veins tended to be low in patients with thrombosis, a finding suggesting a relationship between low vascular fibrinolysis and the occurrence of thrombosis.

The present investigation concerns the localization and estimation of the fibrinolytic activity in biopsy specimens of veins from a large number of patients with thrombosis of the limbs.

MATERIAL AND METHOD

The material consisted of 151 biopsy specimens of veins from 58 patients being followed up because of superficial thrombophlebitis and/or deep venous thrombosis of the arm and/or leg. 51 vein specimens from ten ambulant patients about to undergo biliary surgery and 27 specimens from nine healthy subjects served as controls. The material included the six thrombotic patients and the eight volunteers reported previously (4). The diagnoses of superficial thrombophlebitis and deep venous thrombosis were based on the classical clinical symptoms; the diagnosis of deep venous thrombosis was verified by phlebography in 23 of 33 patients. As a rule the biopsy specimens were obtained under local anaesthesia from the great saphenous vein at the level of the medial malleolus and from a superficial vein of the forearm when palpation revealed occluded veins. Additional specimens were excised from the ipsilateral great saphenous vein but at higher levels and from apparently normal veins in the saphenous area. The material examined is summarized in Table I.

The veins were examined by a modification of the fibrinolysis autography technique of Todd (6). As known this method consists in incubating sections of tissue in contact with a thin film of fibrin rich in plasminogen. During the incubation fibrinolysis occurs at the active sites of the section and on subsequent staining, these sites appear as white empty areas surrounded by undigested fibrin. The method has now been elaborated to permit semiquantitative measurement of the fibrinolytic activity of the sections and thereby also comparison of the activity of different samples. The strength of the fibrinolytic activity of the veins was estimated in the following way. Four fibrin slides were prepared for each specimen and incubated at 37°C for 0, 5, 10, 20 min respectively. Three fairly distinct degrees of fibrinolysis were recognised, namely *grade I* macroscopical punctate areas of lysis in most of the sections, *grade II* gross lytic areas of irregular outline and sometimes confluent, *grade III* dissolution of most or all the fibrin in contact with the sections. A *grade I* slide was allotted 1 point, a *grade II* slide 2 points and a *grade III* slide 3 points. The total number of points scored by the set of four slides was taken as a measure of the fibrinolytic

Table I Biopsy specimens studied

	No of subjects	Right arm	Left arm	Right leg	Left leg
Patients with thrombophlebitis	25	10	7	33	16
Patients with thrombosis	17	6	7	12	13
Patients with thrombosis and thrombophlebitis	16	12	7	13	15
Controls	19	12	10	11	10

activity of the same. Both the method and the reagents used are described in detail elsewhere (5). The rank-sum test of Wilcoxon was used to compare the strength of the fibrinolytic activities of different groups of veins.

RESULTS

Controls

All the veins were microscopically normal. Most of the fibrinolytic activity was confined to the vasa vasorum of the adventitia. The media contained a smaller number of lytic areas while the intima was only rarely active. Arm veins were more active than leg veins (Table II).

Thrombosis and Thrombophlebitis

Some biopsy specimens were too small to allow simultaneous processing by standard histological techniques. The microscopic evaluation of the pathological material was therefore somewhat crude. It was however possible to separate these

Table II Fibrinolytic activity in arbitrary units of limb veins from healthy subjects and patients with thrombosis and/or thrombophlebitis

Figures within parentheses denote the number of specimens

	Patent veins	Veins occluded by compact thrombi	Veins containing canalized thrombi
Arms			
Controls	7.73 ± 1.81^a $2.5-11^b$	(0) (27)	(0)
Patients	6.09 ± 2.21 $0-9^b$	1^a (1) (48)	(0)
Legs			
Controls	4.69 ± 2.11^a $1-8.5^b$	(0) (21)	(0)
Patients	2.53 ± 2.37^a $0-8.5^b$	0.46 ± 0.09^a (61) $0-3^b$	3.91 ± 2.71^a (25) $0-8^b$ (16)

^a Mean \pm s.e. ^b Range

veins into two main groups: one with patent veins and one with thrombosed veins.

Patent veins

In the patent veins of normal appearance the intramural distribution of the fibrinolytic activity was the same as in those from subjects without thrombosis. An illustrative example of this pattern of activity is given in Fig 1. As in the controls, arm veins were more active than leg veins (Table II). The mean fibrinolytic activity of patent arm and leg veins of patients with throm



Fig 1 Unaffected leg vein of a patient with thrombosis. Numerous lytic areas relate to the adventitia and the media. Incubation time 18 min. Magn. $\times 12$.



Fig 2 Saphena magna filled by a compact thrombus. In spite of the prolonged incubation time (60 min) there is no fibrin digestion. The arrow points to an inactive vasa vasorum in the adventitia. Magn. $\times 20$.

bosis was lower than that of the veins of the controls. The difference was highly significant for the veins of the leg ($P < 0.001$) but not significant for the veins of the arm ($P = 0.06$). Of the 61 patent leg veins from the thrombotic patients 15 (about 25%) were completely inactive.

Thrombosed veins

This group was also divided according to the presence or absence of canalizing vessels in the thrombus.

Veins occluded by compact thrombi. The fibrinolytic activity of these veins was negligible

(Table II). Only one arm vein was occluded. Its activity was 1. Of the 25 specimens from the leg as many as 19 had no demonstrable fibrinolytic activity. A specimen of a thrombosed vessel showing no activity even after prolonged incubation is shown in Fig 2. In the few thrombosed veins in which activity was demonstrated it was confined to the vasa vasorum in the adventitia and media. The thrombi themselves were inactive although lysis due to disrupted cells, presumably leucocytes, was occasionally observed.

Veins containing canalized thrombi. On canalization of the thrombus fibrinolytic activity re-



Fig 3 Occluded saphena magna. A single area of fibrinolysis is produced by a newly formed vessel sprouting from the intima. The other newly formed vessels are inactive. Incubation time 10 min. Magn. $\times 30$.



Fig 4 Saphena magna containing a canalized thrombus. Numerous lytic areas related to the canalizing vessels. Incubation time 10 min. Magn $\times 20$

appeared and was then stronger than in patent veins (Table II). The difference however was not significant ($P=0.07$). Canalization began from active capillary buds some of which could be seen growing from the intima (Fig 3). Canalized vessels usually possessed high fibrinolytic activity (Figs 4 and 5) but in some cases inactive canalizing vessels were observed.

DISCUSSION

The present observation that the walls of veins occluded by thrombi contain practically no fibrinolytic activators strengthens the belief that

these enzymes are important in dissolving intravascular clots. The lack of activity of thrombosed veins may tentatively be explained by the assumption that the pre-occlusion plasminogen activator in the vessel has been consumed by attempted physiological thrombolysis or that the pre-occlusive fibrinolytic activity of the vein walls was low and facilitated the development of the thrombus. The latter possibility is supported by the finding of abnormally low fibrinolytic activity in unaffected veins from patients with thrombosis and secondly by the observation that the fibrinolytic activity of the blood after venous stasis is substantially lower in patients with

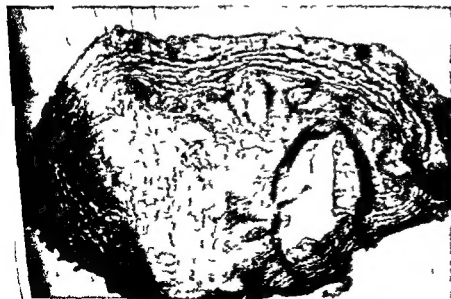


Fig 5 Major canalizing vessel producing a large area of fibrinolysis. Incubation time 5 min. Magn $\times 18$

thrombosis than in controls (2-4). As known stasis is believed to liberate fibrinolytic activators from the vessels (1). It seems justified to consider the possibility of a decreased content of fibrinolytic activators of the vessel walls as a contributory factor in the causation of thrombosis. Whether the decreased fibrinolytic activity of the veins of thrombotic patients is a primary disorder or secondary to some other disturbance must await further research.

The activity observed in the canalizing vessels is in agreement with the findings of Todd in autopsy material (7) and of Kwaan and Astrup in the rat (3).

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CYTOMEGALOVIRUS INFECTION AMONG RENAL ALLOGRAFT RECIPIENTS

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Abstract A comprehensive serologic virologic and histologic study has been made of the incidence and clinical significance of cytomegalovirus infection among 43 renal allograft recipients. Material adequate for investigation was available from 36 patients and evidence of infection was found in 33 giving an incidence of 91%. In the majority of patients infection occurred during the first two months after transplantation. Most infections were clinically inapparent, but in four patients a febrile illness associated with a relative lymphocytosis was seen which it seems reasonable to assume was caused by infection with cytomegalovirus. Cytomegalovirus pneumonia based on the findings of cytomegalic cells in the lungs was seen in 7 of the 17 autopsied patients. Although there was extensive cytomegalovirus pneumonia in three patients death was not considered to have been caused by or indeed significantly contributed to the infection, as all had a bacterial pneumonia as well.

Since 1963 the frequent occurrence of cytomegalovirus (CMV) infection among kidney transplant recipients has been well documented in several reports from two transplantation centers in the United States: Boston and Denver. A characteristic feature of CMV infection is the presence of large cells with hypertrophied nuclei containing intranuclear inclusions, the so-called cytomegalic cells in tissue infected by the virus and the early studies of CMV infection were based on autopsy findings. Hill et al (6) in 1964 mentioned the high incidence of CMV infection in connection with a general discussion of opportunistic infections observed in patients treated with immunosuppressive and cytolytic agents. Hedley Whyte and Craighead (5) confirmed this finding and in addition they were able to isolate CMV from the lungs of a patient with severe pneumonia in which cytomegalic cells had been found on histologic examination. In 1965 Rif-

kind (12) reported virologic studies in three patients with pulmonary cytomegalic cells and noted in addition, an increase in CMV complement fixing (CF) antibodies in kidney transplant recipients. Kanich and Craighead (7) found that it was often possible to isolate CMV from the urine and oropharyngeal secretions of patients after transplantation. These investigators have shown that CMV infection is frequently generalized as the virus may be isolated from several organs at autopsy. Cytomegalic cells have also been found in many parts of the body including the central nervous system (14) but these cells are unusual in organs other than the lungs in kidney transplant recipients.

An analysis of the findings from both American centers was published in 1967. Among 51 autopsied cases Rifkind et al (13) found cytomegalic cells in 52% while cytomegalovirus was demonstrated in 17 of 26 living recipients. These patients had clinical symptoms of pneumonia in association with a rise in CMV CF antibody. In all of the autopsied patients with pneumonia in whom cytomegalic cells were found in the lungs other infectious agents usually bacteria, were always present. Craighead et al (3) studied 41 renal allograft recipients using serologic and virologic techniques and found evidence of CMV infection in 73% of patients surviving one or more months after transplantation. The occurrence of the infection did not correlate with a well-defined clinical syndrome even though generalized CMV infection was found in some patients at autopsy. These investigators also mention that infection may be either primary or the result of reactivation of latent infection.

Finding on autopsy of cytomegalic cells in the lungs and renal graft of one of the first patients in the Aarhus series (10) stimulated our interest in this problem. The object of this investigation has been to evaluate the extent and significance of CMV infection among recipients of a renal allograft in the Aarhus series.

The studies which will be reported here began in November 1966 and were prospective as well as retrospective.

MATERIAL AND METHODS

Originally only a prospective investigation was planned. All patients transplanted in the fall of 1966 and thereafter were studied serologically and virologically at monthly intervals for the presence of CMV infection. Later it proved possible to include several patients transplanted earlier. This retrospective study included both histologic and serologic investigations.

Patient material

Forty-three patients, 21 women and 22 men, with an average age of 31 years have received renal transplants at this Center from April 1964 to the 14th of February 1968. Twenty of these patients have died as of May 1968.

Immunosuppressive therapy

azathioprine 2-4 mg/kg body weight, was used from or before the day of transplantation in all cases. Prednisone 100 to 150 mg/day was started at the first sign of a rejection crisis and thereafter slowly reduced to a maintenance dose of 10 to 20 mg/day unless renewed signs of rejection appeared whereupon the dose was again increased. During the period covered by the prospective study (November 1966 to May 1968) the average daily dose of prednisone given has been 143 mg/kg body weight during the first post-transplant month and 0.61 mg/kg and 0.37 mg/kg during the second and third month. Actinomycin C, 0.005 µg/day was given at the time of a rejection crisis in some patients and was in some cases supplemented with 150 r X-irradiation.

Virus isolation

Human embryonic fibroblasts from muscle (HEM) or lung (HEL) were used for virus isolation and the production of virus antigen. Cell cultures were prepared from embryonic tissues after treatment with a solution of 0.01% trypsin and grown in one liter Roux Flasks. Tissue cultures were used from passages 3 to 30. Eagle's basal medium (EBM) supplemented with 10% calf serum containing 200 IU penicillin and 60 µg streptomycin per milliliter was used as growth medium, while EBM with 2% calf serum and antibiotics served as maintenance medium. 100 µg kanamycin and 4 µg mycostatin per milliliter were added to cell cultures employed in virus isolation to prevent the growth of unwanted microorganisms. Culture medium was changed three times a

week. Cell cultures in roller tubes or 200 ml flasks were used for virus isolation. All cultures were incubated at 37°C. Oropharyngeal washings, using culture medium without antibiotic urine and blood were cultured for virus. The technical procedure employed was modified during the study. Originally 0.3 ml urine or throat washing was inoculated onto culture tubes after storage for one hour at 4°C. Later only freshly voided urine was either added directly to cell cultures (1 ml per flask) or mixed with equal parts EBM containing 0.2% bovine serum albumin at 37°C and the pH adjusted to 7.0 with a 2.8% solution of sodium bicarbonate. Two ml of this mixture was added to a culture flask and 40 ml of the rest was centrifuged at 60 000 g for two hours in a Spinco L-2 ultracentrifuge using Rotor L. 90. Nine tenths of the resultant supernatant was discarded and the rest, together with the sediment, was inoculated onto a flask culture at times the same as that to which freshly voided urine had been added. Blood was cultured for CMV by placing 10 ml fresh heparin-stabilized blood in an incubator at 37°C for one hour allowing the red blood cells to settle out. The plasma was then centrifuged at 1000 g for 15 min, the supernatant discarded and the sediment added to a culture flask after suspension in a few drops of phosphate buffered saline (PBS). Autopsy tissue was ground in a mortar with sand and PBS added to make a 10% suspension. After centrifuging at 1500 g for 15 min one ml of the supernatant was placed in a culture flask. Culture medium was changed 3 to 5 hours after inoculation.

Virus identification

Tissue cultures were examined at weekly intervals for cytopathic effect for 6 to 8 weeks. Cultures not demonstrating cytopathic effect were regarded as negative and discarded. Positive cultures were trypsinized with a 0.1% trypsin solution after cytopathic effect had developed sufficiently. One third of the cells were transferred to a new culture flask, another third were placed in a Petri dish containing three cover glass cultures, and the rest were frozen at -70°C. Cover-glass cultures were incubated in an airtight box under 5% CO₂. On the appearance of cytopathic effect, one cover glass culture was fixed with Bouin's solution stained with hematoxylin-eosin and examined microscopically for the presence of nuclear and cytoplasmic inclusions. The two other cultures were used in an immunofluorescence study using Coombs indirect technique. One known negative and one known positive convalescent serum together with the patient's own serum were employed as the primary sera. The different parts of the cover glass cultures, and a fluorescent anti-conjugated antihuman globulin produced in rabbits served as the secondary serum. All sera were diluted 1:10.

In some cases a CF antigen was prepared from a flask culture after the first passage in order to investigate the antigenic characteristics of the newly isolated strain.

Serological tests

Antigen was prepared by inoculating 1 liter Roux flasks containing human embryonic fibroblasts with 1 × 10⁶ PFU (plaque forming units) of CMV strain AD 169 (the

strain supplied by Dr Gun Carlstrom, Karolinska Sjukhuset, Stockholm) After the development of diffuse cytopathic effect, which took 1 to 14 days, the medium was decanted and 5 ml sterile water added Cells were shaken or scraped free and the cell suspension freeze-thawed twice Cell debris was removed by centrifugation and the antigen containing supernatant stored in sealed glass ampules at -10°C until used.

A standard microtiter CF test (Cook Engineering, Co) was used employing 2 units of antigen, 1 / units of complement and 4 units of hemolysin Antigen, serum and complement were incubated for 11 hours at 4°C . After warming to 37°C the hemolysin system was added and the specimen incubated for 30 min at 37°C 25% hemolysis served as end point on titration All sera were first tested in a 1:4 and 1:8 dilution against both CMV antigen and an antigen prepared from a non-infected cell culture according to the method described above Sera were also tested for anticomplementary activity Positive sera were then titrated against CMV antigen in doubling dilutions from 1:2 to 1:256 together with a serum with known titer All sera from each patient were parallel titrated upon completion of the follow-up period.

Sera from patients in the prospective study were primarily tested after 1 to 3 days at 4°C and then stored at -20°C for later parallel titration Sera from most of the patients in the retrospective study had been stored for 1 month to 4 years at -20°C a few sera, however had been preserved by lyophilization All sera were inactivated at 56°C for 30 min.

In studying neutralizing (NT) antibody the plaque reduction test of Plummer and Benayesh-Melnick was used (11) 7.5×10^4 cells suspended in growth medium were grown to confluence in plastic Petri dishes, 6 cm in diameter in a humidified 5% CO_2 incubator Serum diluted 1:2, 1:10, 1:50 and 1:250 with TRIS-buffer was mixed with a cell free virus suspension of strain AD 169 and incubated for 1 hour in a waterbath at 37°C After replacement of the culture medium by 0.3 ml TRIS-buffer 0.2 ml from each dilution of the serum and virus mixture was inoculated onto two dishes and the cultures again placed at 37°C for one hour with tilting of the dishes every 15 min Thereafter the serum and virus mixture was poured off and the cultures covered by a solid layer of EBM with 10% calf serum, 2% methyl cellulose sodium bicarbonate and antibiotics, and again incubated for 14 days Once or twice during that period a new layer of the above mentioned overlay was added At the end of the 14 days the dishes were placed at 4°C for a few hours, the medium removed and the dishes stained overnight with 0.3% methylene blue The resultant plaques were then counted with the aid of a microscope The titer of the virus suspensions was regulated on the basis of previously made serial titrations so that the virus suspensions yield 40 to 90 plaques with a negative serum and the accuracy and reliability of the test were controlled at each titration by employing known positive and negative sera Serum dilutions showing 80% plaque reduction were used as the end point Titration of virus stocks was also performed by the plaque method.

Histological studies

Lung sections (c 2×2 cm) from the autopsied cases, generally three from each patient, were carefully examined for the presence of cytomegalic cells after fixation in 4% neutral formalin and staining in hematoxylin-eosin.

RESULTS

The following criteria were used in this study in diagnosing CMV infection

- 1 Positive sero-conversion or a fourfold rise of CF antibody on parallel titration.
- 2 Significant rise in NT antibody
- 3 Isolation of virus
- 4 Finding of cytomegalic cells in lung sections on autopsy

Serological studies

For serological determinations c 450 sera were available from 34 of the 43 patients transplanted in the study period.

It was not always possible, particularly in the retrospective group to obtain sufficiently representative material Data on the patients studied the material available and results achieved are shown in Table I No sera at all were available from one patient (in the retrospective study) and from eight others only sera obtained near the time of transplantation could be located Seven of these died soon after transplantation two in the first four in the second and one in the third post transplant month Paired sera from the eighth patient who is still alive were first available from post transplant day 200

At least a fourfold increase in CF antibody or seroconversion was demonstrated in 29 but there was a serious lack of sera from the days immediately after transplantation in one of these patients Details concerning the serologic findings in the remaining 28 patients are given in Tables II and III In ten patients (Table II) no CF antibodies against CMV were detected in the initial sera but in seven of these it was possible to demonstrate a low titer of NT antibodies In 18 patients (Table III) CF antibodies were demonstrable in sera taken before or in a few cases immediately after transplantation There was no relationship between the age of the patients and the presence or absence of CF antibodies as the average age of both groups was 31 years As

Finding on autopsy of cytomegalic cells in the lungs and renal graft of one of the first patients in the Aarhus series (10) stimulated our interest in this problem. The object of this investigation has been to evaluate the extent and significance of CMV infection among recipients of a renal allograft in the Aarhus series.

The studies which will be reported here began in November 1966 and were prospective as well as retrospective.

MATERIAL AND METHODS

Originally only a prospective investigation was planned. All patients transplanted in the fall of 1966 and thereafter were studied serologically and virologically at monthly intervals for the presence of CMV infection. Later it proved possible to include several patients transplanted earlier. This retrospective study included both histologic and serologic investigations.

Patient material

Forty-three patients, 21 women and 22 men, with an average age of 31 years have received renal transplants in this Center from April 1964 to the 15th of February 1968. Twenty of these patients have died as of May 1968.

Immunosuppressive therapy

azathioprine, 4 mg/kg body weight was used from or before the day of transplantation in all cases. Prednisone 300 to 140 mg/day was started at the first sign of a rejection crisis and thereafter slowly reduced to a maintenance dose of 10 to 0 mg/day unless renewed signs of rejection appeared, whereupon the dose was again increased. During the period covered by the prospective study (November 1966 to May 1968) the average daily dose of prednisone given has been 143 mg/kg body weight during the first post-transplant month and 661 mg/kg and 0.37 mg/kg during the second and third month. A cyclosporin C 100 µg/day was given at the time of a rejection crisis in some patients and was in some cases supplemented with 140 r X-irradiation.

Virus isolation

Human embryonic fibroblasts from muscle (HEM) or lung (HEL) were used for virus isolation and the production of virus antigen. Cell cultures were prepared from embryonic tissues after treatment with a solution of 0.01% trypsin and grown in one liter Roux Flasks. Tissue cultures were used from passages 3 to 30. Eagle's basal medium (EBM) supplemented with 10% calf serum containing 200 IU penicillin and 60 µg streptomycin per milliliter was used as growth medium while EBM with 2% calf serum and antibiotics served as maintenance medium. 100 µg kanamycin and 5 µg mycostatin per milliliter were added to cell cultures employed in virus isolation to prevent the growth of unwanted microorganisms. Culture medium was changed three times a

week. Cell cultures in roller tubes or 200 ml flasks were used for virus isolation. All cultures were incubated at 37°C. Oropharyngeal washings using culture medium without antibiotic urine and blood were cultured for virus. The technical procedure employed was modified during the study. Originally 0.5 ml urine or throat washing was inoculated onto culture tubes after storage for one hour at 4°C. Later only freshly voided urine was either added directly to cell cultures (1 ml per flask) or mixed with equal parts EBM containing 0.2% bovine serum albumin at 37°C and the pH adjusted to 7.0 with a 2.8% solution of sodium bicarbonate. Two ml of this mixture was added to a culture flask and 40 to 40 ml of the rest was centrifuged at 60 000 g for two hours in a Spinco L-2 ultracentrifuge using Rotor L 50. Nine tenths of the resultant supernatant was discarded and the rest, together with the sediment, was inoculated onto a flask culture at times the same as that to which freshly voided urine had been added. Blood was cultured for CMV by placing 10 ml fresh heparin-stabilized blood in an incubator at 37°C for one hour allowing the red blood cells to settle out. The plasma was then centrifuged at 1000 g for 15 min, the supernatant discarded and the sediment added to a culture flask after suspension in a few drops of phosphate buffered saline (PBS). Autopsy tissue was ground in a mortar with sand and PBS added to make a 10% suspension. After centrifuging at 1500 g for 15 min one ml of the supernatant was placed in a culture flask. Culture medium was changed 3 to 5 hours after inoculation.

Virus identification

Tissue cultures were examined at weekly intervals for cytopathic effect for 6 to 8 weeks. Cultures not demonstrating cytopathic effect were regarded as negative and discarded. Positive cultures were trypsinized with a 0.1% trypsin solution after cytopathic effect had developed sufficiently. One third of the cells were transferred to a new culture flask, another third were placed in a Petri dish containing three cover glass cultures and the rest were frozen at -70°C. Cover glass cultures were incubated in an airtight box under 5% CO₂. On the appearance of cytopathic effect, one cover glass culture was fixed with Bouin's solution, stained with hematoxylin-eosin and examined microscopically for the presence of nuclear and cytoplasmic inclusions. The two other cultures were used in an immunofluorescence study using Coon's indirect technique. One known negative and one known positive convalescent serum together with the patient's own serum were employed as the primary sera on different parts of the cover glass cultures and a fluorescein-conjugated antihuman γ -globulin produced in rabbits served as the secondary serum. All sera were diluted 1:10.

In some cases a CF antigen was prepared from a flask culture after the first passage in order to investigate the antigenic characteristics of the newly isolated strain.

Serological tests

Antigen was prepared by inoculating 1 liter Roux flasks containing human embryonic fibroblasts with 2.5×10^6 PFU (plaque forming units) of CMV strain AD 169 (the

Table III Results from 18 patients with a fourfold or greater rise in CF antibodies against CMV

Pat no	Initial CF titer	Complement fixing antibody				First serum with highest titer		Latest titer		Living or dead
		Days post transplant between which a four fold or greater rise in titer registered				Titer	Day	Titer	Day	
		Month 1	Month 2	Month 3	Month 4 or later					
T 3	II		15-45			64	45	"		D
T 7	32	(-2)-17				256	17	32	314	D
T 9	4		23-60			64	60	<4	320	D
T 11	16				78-100	128	125	"		D
T 12	32				98-126	256	126	16	956	L
T 15	4			47-77		18	77	64	778	L
T 16	8		21-46			128	63	32	736	L
T 18	4			56-74		256	92			D
T 20	4	(-1)-22				256	22	8	515	L
T 23	16				23-198	64	198	64	48	L
T 24	8		27-54			256	116	64	418	L
T 26	8			4-80		64	151	16	456	L
T 27	2			35-76		16	76	<2	448	L
T 30	8	(-13)-28				128	94	128	216	L
T 31	III				108-122	256	122	64	205	L
T 33	2		13-47			64	47	"		D
T 42	4			55-83		16	83	16	93	L
T 43	4	23-6				16	26	16	40	L

No sera available after antibody rise

A graphic illustration of the serologic studies by the CF and NT tests in six patients is given in Figs 1 and 2. In several sera from these six patients NT antibodies against strain AD 169 were demonstrated. These antibodies were found in patients with CF antibody before transplantation and in patients 23, 24 and 26 a parallel increase in CF and NT antibodies was seen but not in patient 27.

Patients 28 and 29 who did not have detectable CF antibodies at the time of transplantation did not have NT antibody either. A marked rise was however demonstrated in these patients but it was somewhat delayed in relation to the rise in CF antibody as the first sera to show a rise in CF antibody did not contain demonstrable NT antibody. In another four kidney transplant patients we have recognized a rise in NT antibody against AD 169 after increase in CF titer.

A significant decrease in CF antibody titer against CMV was often registered in the first sera after transplantation such as that seen in patients nos 26 (Fig 1) and T 11 (Table V). A similar phenomenon was seen in some deceased patients with detectable CF antibody before

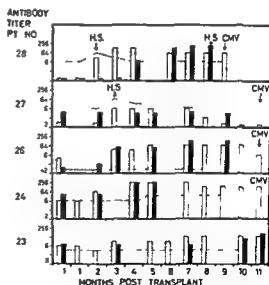


Fig 1 Studies of cytomegalovirus complement fixing and virus neutralizing antibodies in five patients after kidney transplantation. White columns represent titer of complement fixing and black on virus neutralizing antibodies against cytomegalovirus. The broken line depicts the titer of herpes simplex virus complement fixing antibody. Isolation of cytomegalovirus (CMV) and herpes simplex virus (H.S.).

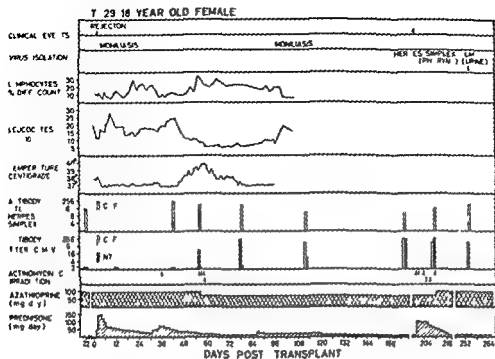


Fig. 7. Post-transplant course of patient T 29 with a febrile illness in association with rise of complement

fixing and virus neutralizing antibodies against cytomegalovirus.

transplantation and no rise in the post-transplant sera.

Also illustrated in Figs 1 and 2 are the results of an investigation of CF antibody against Herpes simplex virus in the same sera. A significant increase was seen in three and Herpes simplex virus could be isolated from throat washings from all three. There was no CF antibody to Herpes simplex virus in any of the sera from patient 26. Generally there was no corresponding increase in these antibodies after transplantation similar to that seen with CMV.

Virus isolation

Attempts at isolation of CMV from renal graft recipients by inoculating urine, throat washings and lung suspensions onto tissue culture may involve a great deal of tedious work. The main reason for this is that tissue cultures often become contaminated by microorganisms resistant to the antibiotics usually used in tissue culture media. We have attempted to solve this problem by adding to the culture media antibiotics to which the bacteria usually identified as *Pseudomonas* sp. were found to be susceptible according to sensitivity tests. In general Kanamycin

(100 µg/ml) proved sufficient to prevent bacterial contamination. Occasionally it was necessary to use chloramphenicol (50 µg/ml) or colimycin B (50 units/ml) in addition in order to carry the cultures until either a viral effect could be observed or the cultures discarded as negative. This circumstance and the fact that some of the inocula contained cytotoxic substances resulted in destruction of some or all of the tissue cultures in about half of the attempts at virus isolation.

Only the isolation attempts in which cultures were kept alive for at least 30 days or in which viral growth was noted are included in this report. Virus isolation has generally not been attempted among patients in the retrospective group.

Longitudinal virologic studies were conducted in one group of ten patients for up to 10 months after transplantation and it was possible in these patients to carry out 88 virus isolation attempts by inoculation of urine and throat washings onto roller tubes. All 44 urine isolations were negative but Herpes simplex virus was isolated from throat washings six times in five patients.

Later flask cultures inoculated with freshly voided urine or ultracentrifuged urine were used

Table IV Results of investigations for CF antigens of isolated strains from kidney transplant recipients

a	Titer AD 169	Isolated strains				
		T 33 lung	T 29 urine	T 28 urine	T 36 urine	T 38 urine
negative	<2	<2	<2	<2	<4	<4
negative	<4	<2	<2	<2	<4	<4
negative	<4	<2	<2	<2	<4	<4
live	<4	<2	<2	<2	<4	<4
negative	<4	<2	<2	<2	<4	<4
29 day 27	<4	<2	<2			
36 day 9	<2	<2	<2	<2	<4	<4
36 day 57	64				32	32
36 day 79	32				16	16
36 day 86	32	32	64	32		
live	32	32	64	32	3	32
positive	8	16	8		16	16
live	16	16	32	32		
live	128	256	256			
live	64	256	128			
live	32				32	32

130 isolation attempts from 15 patients CMV was recovered 16 times from 11 patients in this way and what were probably adenoviruses were isolated from three patients during one month in the fall of 1967. We did not isolate CMV from the urine of patients in whom a rise of CF titer had not been demonstrated. In one patient virus was isolated 26 days after sero-conversion. In another viruria was first demonstrated twice about 300 days after rise of the titer at a time when CF antibodies were no longer detectable in the patient's serum. Virus was found in the urine from another patient 717 days after antibody rise.

CMV was isolated from the peripheral blood once on 11 attempts from seven patients. This patient died on post transplant day 83 without any rise in CF antibody but there was a titer in the initial serum. The inoculation of a suspension of lung tissue onto flask cultures from two patients in whom cytomegalic cells had been found on histologic examination of the lungs resulted in the isolation of CMV in both cases.

The typical cytopathogenic effect of CMV was generally seen in flask cultures 11-27 days after inoculation with infective material.

Virus identification

The strains of CMV isolated in this study possessed characteristics similar to those of strain AD 169. All 19 formed elongated foci composed of round clear cells in primary as well as in

passage culture. These foci spread very slowly in spite of prolonged incubation and diffuse cytopathic effect was as a rule first seen in the passage cultures.

Nuclear as well as cytoplasmic inclusions were seen as part of the cytopathic effect of the isolated strains in hematoxylin-eosin stained preparations.

Immunofluorescence studies of these strains revealed a very characteristic picture corresponding to that produced by cultures inoculated with AD 169. Using a known convalescent serum (CF titer 64 NT titer 200) and serum with antibodies against CMV from the patient from whom the virus had been isolated fluorescence was seen in the focal changes in tissue cultures demonstrating the cytopathic effect but not in the non affected cells. There was only a very weak fluorescence of these foci when the routinely used negative serum (CF titer <2 NT titer <2) was employed and neither this serum nor the positive sera produced specific fluorescence in the non inoculated cultures.

The positive standard serum resulted in a very characteristic fluorescence pattern in the cells infected by all the isolated strains. The nuclear inclusions which were of varying size according to the degree of infection in affected cells stood out very sharply while the rest of the cells especially the cytoplasm fluoresced much more weakly.

The pattern of fluorescence in the cultures

Table V. Studies in seven patients with cytomegalic cells in the lungs

Pat no	Histologic diagnosis	Died post transplant day	Serology CF antibody	
			Day	Titer
7	Sporadic cytomegalic cells	335	-2	32
			+17	256
			+757	128
			314	32
8	Focal cytomegalic alveolar pneumonia	35	+19	16
			+34	16
11	Several cytomegalic cells diffuse in the lungs	175	-54	16
			-9	8
			-78	8
			+100	64
			+125	128
19	One cytomegalic cell	34	-30	64
			7	32
			14	32
29	Sporadic cytomegalic cells	329	-77	<2
			-41	2
			-36	32
			-78	256
			-303	128
37	Focal cytomegalic alveolar pneumonia	39	-2	7
			-27	2
			-36	<2
7	Extensive diffuse cytomegalic pneumonia	47	+13	2
			47	64

treated with positive serum from the patient from whom the strain had been isolated was usually of similar appearance but sera from some of the patients produced a strong fluorescence of the nuclear membrane and the cytoplasm while the nuclear inclusions fluoresced only weakly or not at all.

Studies of the CF antigen of five of the newly isolated strains were carried out with several screenings and parallel titrations using a series of known positive and negative sera some of which had been obtained from patients with known seroconversion. The results of these studies are shown in Table IV.

All the strains of Herpes simplex virus isolated in this study could be neutralized by an immune serum produced in rabbits.

Identification of three virus strains which apparently belong to the group of adenoviruses is not yet complete. These strains grow slowly in HEL and HEM cells but rapidly produce a dif-

fuse cytopathic effect in HeLa cultures. Basophilic nuclear inclusions typical of adenovirus are formed and the virus is ether resistant.

Histological studies

Lung sections were available from 17 patients. Cytomegalic cells were found in seven.

Cytomegalic cells are very characteristic. They are significantly enlarged, 30 to 40 μ in diameter and contain a large round nucleus which is almost completely filled by a homogeneous inclusion body. Between this body and the nuclear membrane a narrow clear halo is usually seen. Cytoplasmic inclusions of the type seen in virus infected tissue cultures were not seen in the cytomegalic cells observed in this series.

The distribution of cytomegalic cells in the lungs varied greatly from patient to patient. In some patients several cells were found in a single small area while in others they were seen diffusely throughout the lungs. As acute and chronic changes (broncho-pneumonic vascular with bleeding etc.) are very common in the lungs from kidney recipients it may be rather difficult to evaluate the possible significance of the CMV infection. In addition other studies (7) have shown that there is no direct relationship between the number of cytomegalic cells and the amount of virus.

By reviewing the individual lung sections and by comparing sections however information can be obtained that gives an impression of the severity of the infection. In patient T32 cytomegalic cells in rather large numbers were found in association with a single alveolus or several closely lying alveoli. The cells lay along the alveolar septa like large epithelial cells. In another case (T8) likewise with marked focal changes a lymphocytic infiltration was seen which masked the structure of a dozen alveoli. Several typical cytomegalic cells were found among the lymphocytic cells. In a third patient (T33) cytomegalic cells were seen in large numbers along the alveolar septa with one or more cells in almost every alveolus. Only single isolated cytomegalic cells were found in the other four patients. In T19 only one was found.

The histologic findings together with the time of death after transplantation and the results of the serologic tests from the seven patients are given in Table V. As can be seen CMV pneu-

monia localized or diffuse was found in patients who died relatively soon after transplantation (35 to 47 days). The other patients in whom only single cytomegalic cells were seen diffusely spread throughout the lungs lived somewhat longer (125 to 335 days). CF antibody was present at death in six of the seven patients and in four an antibody rise was demonstrated in the post transplant period (Table V).

The ten patients in whom cytomegalic cells were not found in the lung sections died at varying periods after transplantation: two in the first month, two in the second, two in the third, two in the fourth, one in the ninth and one in the twentieth month post transplant. Serologic studies in six of these patients showed a significant rise of antibody titer in four and no demonstrable rise in two. No sera were available from the other four patients who died from 18 to 81 days after transplantation.

The results of this study indicate that CMV can produce specific pneumonic changes. Changes are particularly marked in the patients who died in the period after transplantation in which the majority of the patients in our series (90% in the prospective study) demonstrate a rise in CF antibody. A clinical syndrome was seen during this period in three patients in the prospective study that suggests a generalized infection (see below). Cytomegalic infection may apparently take a chronic course in the lungs as cytomegalic cells were found in the lungs of one patient (T 29) 273 days after a significant rise in CF antibody had been demonstrated (see Table V).

Clinical symptoms in connection with CMV infection

In none of the seven patients in whom cytomegalic cells were found in the lungs on autopsy was death considered to have been caused or significantly contributed to by the CMV infection. Six of these patients had symptoms and radiologic signs of pneumonia at death but in every case autopsy findings revealed clearcut evidence of extensive bacterial pneumonia. Distinct nodular densities were seen on X ray in two of these patients but autopsy did not provide sufficient information to determine whether these densities were caused by the CMV infection or by the bacterial pneumonia.

Patients in the prospective study were questioned and examined as soon as a significant rise in CF antibody was ascertained. Five of these 17 patients developed clinical symptoms within the month preceding antibody rise which may have been caused by the CMV infection. Two of them had only a few days of unexplained low grade fever but the other three presented a clinical syndrome with prolonged fever, malaise, relative lymphocytosis and slight leucopenia. Two had a dry cough at the beginning of the illness and increased pulmonary vascular markings were seen on X ray in one. The case histories of the three patients will be briefly reviewed.

T 29 (Fig. 2)

An 18-year-old girl with chronic glomerulonephritis who received a kidney from her father in March 1967. The operation was performed without complications. Evidence of a mild rejection crisis appeared on the second post operative day and prednisone therapy was started. There was a more severe rejection crisis in the fifth post operative week with sodium retention, weight increase, decreasing renal function and significant lymphocyturia. This crisis, as the first, responded to prednisone therapy. An average daily dose of 135 mg prednisone per kg body weight was given during the first post transplant month and 0.91 mg/kg and 0.39 mg/kg during the second and third month. Forty-three days after transplantation the patient developed fever which continued until the seventy-first day. Fever was inconstant; the temperature was almost normal during the early morning and between 38° and 40° C in the evening. There was headache, mild nausea and malaise during the first week of fever. A dry cough was present but the chest was clear to percussion and auscultation. Chest X ray revealed increased pulmonary vascular markings; films taken before and after this febrile illness were interpreted as normal. There was a fall in leucocyte count together with a relative lymphocytosis. Serum glutamic oxalacetic transaminase measured 46 units 9 days after the onset of fever and was below 30 units a week later. There was no evidence of graft rejection during this period other than fever and graft function remained good throughout with a creatinine clearance of 50 to 80 ml/min. Serological tests for ornithosis and toxoplasmosis were negative. CMV CF titer two days before fever appeared was less than 2. Two weeks later it was 32, and three weeks thereafter the titer was 256. Virus neutralizing antibody began to rise 78 days after transplantation. It was possible to culture CMV from the urine on days 254 and 262 post transplant. A chronic vascular allograft reaction developed nine months after transplantation which proved impossible to control and the patient died in uremia 3.9 years after renal allograft transplantation. Cytomegalic cells were found in the lungs on autopsy and it was possible to culture from lung tissue.

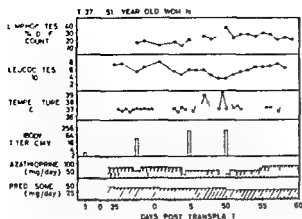


FIG. 3 Post-transplant course of patient T 37 with a febrile illness in association with rise of complement fixing antibodies against cytomegalovirus

T 37 (Fig. 3)

A 41-year-old woman with chronic pyelonephritis who received a kidney from her sister in November 1967. Minimal signs of rejection in the form of fever and graft tenderness appeared on the second post-operative day and prednisone therapy was started with 150 mg/day. Prednisone was thereafter reduced stepwise the patient receiving in the first three months after transplantation an average daily prednisone dose of 1.20 mg/kg, 0.6 mg/kg and 0.33 mg/kg respectively. Graft function was good during the entire post-operative course with a clearance of 40 to 60 ml/min and there were no further rejection crises. The patient became febrile on the forty-fifth post-operative day and remained so during the following six days with almost normal temperature in the morning and 38 to 39.5 °C in the evening. At the start of the fever the patient complained of a dry cough but pulmonary stethoscopic findings and chest X-ray were normal. From post-transplant day 47 to 53 there was slight leucopenia 3500 to 5000 leucocytes/ μ l accompanied by a slight relative increase in the percentage of lymphocytes on differential count. The peripheral blood did not contain typical Downey cells or an increased percentage of monocytes but most of the lymphocytes were slightly atypical with increased basophilia and about 10% of them had nuclei containing nucleoli. On day 50 the serum glutamic oxalacetic transaminase was 60 units, five days later it was less than 35 units. CMV CF antibody five days before transplantation was <2, on post-transplant day 31 it was 3⁺. Titer has remained elevated through day 174 (see Table II). Serologic tests for toxoplasmosis, influenza, ornithosis and infectious mononucleosis (Paul Bunnell) were all negative.

T 40 (Fig. 4)

A 21-year-old woman with congenital hypoplastic kidneys who received a kidney from her mother in January 1968. There were no operative complications but the post-operative course was characterized by ureteral problems and urinary tract infection. There was a very mild rejection episode two days after transplantation

with fever but no reduction of graft function. Prednisone was started at that time and the average daily dose was 1.75 mg/kg, 0.71 mg/kg and 0.47 mg/kg during the first three post-transplant months. There was no further evidence of graft rejection and renal function has remained excellent throughout the post-operative course with a creatinine clearance of 70 to 80 ml/min. On the 83rd post-transplant day the patient developed fever, chills and malaise after having rhinitis during the preceding week. There were no pulmonary symptoms and physical and X-ray findings were interpreted as normal. Fever which was septic in character—high in the evening low in the morning—lasted for 14 days. This temperature elevation was at first thought to be due to a urinary tract infection but streptomycin was without effect and urinary sediment and bacteriology were the same before the fever as after. Leucopenia with white cell counts below 5000 cells/ μ l developed on the 88th post-transplant day and continued for about two weeks. Lymphocytosis appeared on day 100 and lasted for 74 days. Monocytosis was not seen. The majority of these lymphocytes which made up from 40 to 80% of the differential count were atypical having the characteristics of the virocytes seen with several viral infections. Serum glutamic oxalacetic transaminase was not elevated. Paul Bunnell ornithosis CF, cold agglutinins, toxoplasmosis CF, Sabin dye test and tests for influenza CF antibodies were all negative. Significant rise in CMV CF antibody was first demonstrated in serum removed on post-transplant day 103 when a titer of 128 was found. Titer on day 95 had been <2. There were three transient episodes of unexplained fever on post-transplant days 133, 146, 155, 165 and 169. In most cases chills preceded the fever which was accompanied by a sensation of nasal stuffiness. A fall in leucocyte count to about 4000/ μ l with relative lymphocytosis was seen from post-transplant day 150 to 154.

On review of the case records from the 12 patients in the retrospective study in whom significant antibody rises were demonstrated only one patient was found who presented signs and symptoms that may have been due to CMV infection. This was a 32-year-old man T 10 in whom seroconversion took place between post-transplant day 9 and 45. This patient had fever from day 15 to 43 which at the time was thought to be the result of esophageal moniliasis.

DISCUSSION

In the work reported here we have attempted to study the problem of CMV infection in kidney transplant recipients as comprehensively as possible. In general we have employed the same methods of study as used by previous investigators (3, 13) but we have had more serum samples available from each patient and in addition we have undertaken studies of NT antibody

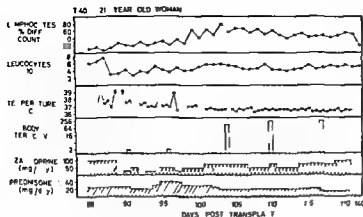


Fig 4 Post transplant course of patient T 40 with a febrile illness in association with rise of complement fixing antibodies against cytomegalovirus.

Experience with certain aspects of the techniques employed will be discussed briefly. It became quite evident that flask cultures were superior to cultures in roller tubes for the isolation of CMV from the urine and blood of adults. Morphologically the tube cultures were generally good after 60 days of incubation but only once was it possible to isolate CMV in tube cultures from an adult. In this case it proved necessary however to transfer the virus to flask culture in order to save the isolated strain. In flask cultures cytopathic effect can usually be observed in the course of one month after inoculation with virus-containing material and diffuse cytopathogenic changes often develop rapidly after passage.

The immunofluorescence reaction has been a significant help in the diagnosis of CMV strains. This test was not absolutely necessary as all of the isolated CMV strains formed characteristic nuclear and cytoplasmic inclusion bodies as part of the cytopathogenic changes. As a serologic technique the immunofluorescence reaction is superior to the study of a strain's CF antigen because it can be carried out less than a week after the first passage of a strain while it takes one to several months to produce a potent CF antigen from a newly isolated strain.

Measuring the titer of NT antibodies by the plaque reduction technique of Plummer and Benjesh Melnick (11) has as far as we know not previously been used in studies of CMV infection. Our experience with this test has been good. We have studied paired sera from ten kidney transplant recipients as well as from three previously healthy adults with CMV infection (15)

and were able to demonstrate virus NT antibodies against strain AD 169 in all patients. In all but one we were able in addition to demonstrate a significant rise in NT titer which in some patients did not begin until 1 to 2 months after the rise in CF titer had taken place.

Preliminary unpublished work on the relationship between CF and NT antibodies in sera from about 50 children and adults has shown that it is generally possible to demonstrate NT antibodies against AD 169 in sera with CF antibodies by this technique. In some CF negative sera NT antibodies were found. These findings are similar to those of Carlstrom (1) and Carlstrom et al (2) using a tube NT test with AD 169.

We have not as yet had the opportunity to test for the presence of NT antibodies against CMV strains other than AD 169.

CMV infection is characterized by the fact that patients excrete virus in saliva and urine. In this study we have mainly concentrated on the study of viruria but we did demonstrate in one patient that virus can be isolated from the blood prior to antibody rise. Isolation of CMV from the blood has been reported previously from patients with leukemia (4) and postperfusion CMV infection (9). It may also be a useful test in the diagnosis of CMV infection in patients treated with immunosuppression. Herpes simplex virus was isolated several times but the level of CF antibodies against this virus did not differ much from the normal. Nor was it specifically influenced by immunosuppressive therapy. Adenoviruses were isolated from the urine of three pa-

tients during a very short period. We have not yet undertaken serological investigations but the isolation of only three strains during this study indicates that this virus does not play as important a role as CMV in renal allograft recipients.

A high frequency of CMV infection in kidney transplant recipients treated with immunosuppressive drugs was documented in 1967 by two groups of American investigators (3, 13). We have found a frequency of about 90%. The infection may appear relatively soon after the start of immunosuppressive therapy although the time of infection varied from patient to patient. In some there was serologic evidence before the end of the first post-transplant month while in others infection appeared during the third or fourth month or even later. In the majority however active infection occurred during the second month after transplantation. It was also in this month that evidence of infection was usually seen in some cases presenting as CMV pneumonia on autopsy in others as a febrile illness. Infection may become chronic either in the form of a protracted lung infection characterized by the presence of cytomegalic cells such as in T 29 in whom cytomegalic cells were found on autopsy 273 days after a significant antiviral rise and as a prolonged viraemia such as demonstrated in case T 16 in which virus was isolated from the urine 717 days after antibody rise.

In general elevated CF antibody titers were seen in these patients for long periods after transplantation but they have disappeared from the serum of one patient (T 27) in spite of persistent viraemia which may perhaps mean that in this patient infection was localized to the graft. Just as Craighead et al. (13) we find that CF antibody titer is higher in transplanted patients treated with immunosuppression than in previously healthy individuals with CMV infection however we do not find as the above mentioned investigators did that it is common for the level of CF antibody to decrease prior to death. In our study we have seen in several patients with CF antibodies in the pre-transplant sera that a fall in titer may occur just after institution of the immunosuppressive therapy prior to rise in titer.

Among the 43 patients studied there was a remarkable number with CF antibodies in their serum before transplantation—about double as many as would be expected for the age groups

involved. In 180 healthy adults between the ages of 25 and 45 years we have found as have others (1) an incidence of about 40%. It is perhaps not surprising that our patients had this higher incidence as all of them suffered from a prolonged illness before transplantation that might have reduced their resistance to viral infection.

In most cases CMV infection in the Aarhus series of kidney transplant recipients appeared to be the result of the reactivation of latent infection. In the pre-transplant serum from only five patients was it impossible to demonstrate CF or NT antibodies against CMV strain AD 169. This suggests that the three who developed antibodies had a primary infection with CMV. One of these patients (T 29) received a kidney from a donor demonstrating a high CF antibody titer and it is possible that she received CMV together with the donor kidney. Other exogenous sources are of course possible.

A characteristic febrile illness was seen in three patients in close association with a rise in CF antibody. None of them had CF antibody before transplantation but two of them had low titers of NT antibody and infection in these two was presumably the result of reactivation of latent infection. In one of the patients (T 29) viraemia was demonstrated several times and cytomegalic cells were found in the lungs at autopsy. Virus has also been isolated from the urine of another (T 37) while this has not yet been possible in the third. This illness with fever, malaise and lymphocytosis has not previously been reported as a manifestation of CMV infection complicating immunosuppressive therapy but such an illness is a common expression of the disease among normal healthy adults (2, 8, 15). Evidence of acute CMV pneumonia was found on autopsy in three patients (T 8, 32 and 33) all of whom had clinical and radiologic signs of pneumonia before death. However autopsy also revealed extensive bacterial pneumonia in all three and therefore it is uncertain what role CMV played in the symptomatology. Two patients (T 29 and T 37) had a dry cough at the start of their clinical illness and perhaps they had a CMV pneumonitis. Slight serum oxalacetic transaminase elevation was seen in cases T 29 and 37 but jaundice was not observed. Nodular densities similar to those described by Riskind et al. (13) were seen on chest X-ray in two of the patients with autopsy evi-

dence of CMV pneumonia but here again it is difficult to ascribe this finding to CMV as both had bacterial pneumonia as well

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ENDOCARDIAL PACING IN ACUTE MYOCARDIAL INFARCTION

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Abstract From 1964 to 1968 seventy seven patients in Ullevål Hospital with acute myocardial infarction were treated with endocardial pacing. Sixty of these patients had A-V block 53 complete and 7 A-V block of second degree

Indication for pacing in the patients with A-V block was Adams Stokes attack in 3 patients, bradycardia in 14 and in 23 prophylactic pacing was performed. Thirty three patients (55%) survived and 7 (45%) died. Mortality was higher in patients with anterior myocardial infarction (69%) than in patients with posterior myocardial infarction (13%). All the patients with both anterior and posterior myocardial infarction died. Patients with bundle branch block had a higher mortality (68%) than patients with narrow QRS complexes (71%).

Two patients died during positioning of the electrode and 11 during treatment with pacemaker. Among the 19 fatalities during pacing 5 were due to ventricular fibrillation, 1 to heart rupture and 13 to shock or cardiac failure.

In 17 patients pacing was started on indications other than A-V block and 13 of the latter (76%) died.

Better results in the treatment of A-V block in acute myocardial infarction will probably be achieved by the use of "on demand" pacemakers in order to avoid pacemaker induced ventricular fibrillation and by prophylactic pacing before serious complications after Adams Stokes attacks or bradycardia have developed.

Complete heart block is reported to occur in about 8% of cases with acute myocardial infarction (5) and the mortality rate is reported to be high figures of 50% and higher are given (3).

Artificial electrical stimulation of the heart is proposed to improve the outlook for these patients. Clinical experience however is still limited and there is no general agreement concerning the practical performance.

Our experience concerning cardiac pacing in acute myocardial infarction is reported in the following.

MATERIAL AND METHODS

Seventy-seven patients with acute myocardial infarction have been treated by endocardial pacing in the period

from May 1964 to August 1968. Fifty three were male average age 61 years, 24 women average age 65 years.

Sixty of the patients had A-V block in 17 patients pacing was started on other indications.

In the first 4 cases a Cournand bipolar electrode in the others unipolar Elema pacing catheters have been used. In 11 cases positioning of the electrode was guided by intracardial ECG recording from the catheter electrode without X ray monitoring, as described elsewhere (1).

Until November 1967 only fixed rate pacemakers were used in most cases the Elema 138 external pacemaker. Pacing voltage was usually 2-3 times the threshold value. In the last year we have also used a ventricular inhibited "on demand" pacemaker.

Patients with A-V block

In 60 patients the indication for pacing was A-V block. Age and sex distribution in this group appear from Table I. Average age of the men was 63 years and of the women 67 years. Fifty three had complete A-V block, seven second degree A-V block. The patients with second degree A-V block all had posterior myocardial infarction. Wenckebach's periods were observed in 11 patients. In 5 patients a progress was seen from second degree to third degree A-V block, two of them had also bundle branch block.

Location of myocardial infarction

If information obtained at autopsy is added to the ECG information, it appears that 13 patients had isolated anterior myocardial infarction, 31 isolated posterior myocardial infarction, 2 lateral myocardial infarction, 8 both anterior and posterior myocardial infarction, 1 anterior and lateral, 1 posterior and lateral and 1 anterior lateral and posterior myocardial infarction (Table II). In 3 patients the location of the myocardial infarction is unknown.

Bundle branch block

Wide ventricular complexes (QRS duration 0.10 sec or more) was seen in 31 patients after the appearance of A-V block. In the period before development of the A-V block bundle branch block was present in 9 patients, not present in 12, and in 10 no ECG recording was available from this period.

Two patients with posterior myocardial infarction had intermittent bundle branch block. In 11 patients with

Table I. Patients with A-V block in different age groups

Age	Male		Female		Sex	
	Total	Deaths	Total	Deaths	Total	Deaths
<40	5	1	0	0	5	1
50-59	12	2	2	1	15	3
60-69	13	8	8	4	21	12
70-79	12	7	5	2	17	9
80+	2	2	0	0	2	2
Sum	44	20	16	7	60	27

anterior of both the posterior and anterior part of septum, bundle branch block developed some hours after the appearance of A-V block. In 29 patients bundle branch block was not observed.

Interval between onset of myocardial infarction and development of A-V block

The interval from the onset of myocardial infarction and development of A-V block was less than 24 hours in 12 patients, 1-2 days in 18, 2-7 days in 9 and unknown in one. Among 13 patients with anterior infarction about one had developed A-V block within 24 hours. Of 25 patients with posterior myocardial infarction two thirds had A-V block within 24 hours (Table 1).

RESULTS

Indication for pacing

The indication for pacing was Adams-Soltes attacks in 23 patients. In nine of these patients the attacks were not recorded on ECG in the remain-

Table II. Mortality related to bundle branch block, location of myocardial infarction and indication for pacing

In brackets: Deaths in the different groups (EBS = bundle branch block)			
All cases	EBS observed 7 (21)	EBS not observed 29 (6)	Sum 60 (27)
<i>Location of myocardial infarction</i>			
Anterior	12 (7)	1 (1)	13 (8)
Posterior	6 (1)	25 (7)	31 (8)
Lateral	1 (1)	1 (1)	2 (2)
Anterior and posterior	8 (1)	0	8 (1)
Anterior and lateral	1 (1)	0	1 (1)
Posterior and lateral	0	1 (1)	1 (1)
Anterior, posterior and lateral	1 (1)	0	1 (1)
Unknown	2 (1)	1 (1)	3 (2)
<i>Indication for pacing</i>			
Adams-Soltes attack	17 (17)	6 (6)	23 (23)
Bradycardia	8 (1)	6 (6)	14 (7)
Prophylactic	6 (1)	17 (2)	23 (3)

ing 14 patients ventricular standstill or bradycardia was registered. Fourteen patients had ventricular bradycardia but no Adams-Soltes attacks. Twenty-three patients with A-V block grade 2 or 3 had neither Adams-Soltes attacks nor bradycardia, and the pacemaker was inserted prophylactically to prevent such episodes. The incidence of Adams-Soltes attacks was greater in patients with bundle branch block (66%) than in those without (21%). Five patients with posterior

Table III. Mortality related to the interval between onset of myocardial infarction and development of A-V block. In brackets: Deaths

	A-V block developed within 24 hours	A-V block developed after more than 24 hours	Interval between onset of infarction and A-V block unknown
Total A-V block	31 (13)	21 (13)	1 (1)
Second degree block only	1 (0)	6 (1)	
All cases	32 (13)	27 (14)	1 (1)
<i>Location of infarction</i>			
(Second degree block excluded)			
Anterior	6 (3)	7 (6)	
Posterior	16 (2)	9 (7)	
Lateral	1 (1)	1 (1)	
Anterior and posterior	6 (6)	2 (2)	
Anterior and lateral	0	1 (1)	
Anterior, posterior and lateral	0	1 (1)	
Posterior and lateral	0	1 (1)	
Unknown	2 (1)	0	1 (1)

myocardial infarction and narrow QRS complexes gave a history of one or more syncope which occurred before the patients arrived in the hospital. One of these patients was unconscious on admission to hospital and died shortly afterwards.

Ventricular fibrillation during pacing for A-V block

Ventricular fibrillation occurred in 11 patients with A-V block. In ten of these patients ventricular fibrillation was observed during effective pacing. Nine had fixed rate pacemakers and one on demand pacemaker. Five died during or shortly after the episodes. Previous thereto most of the patients were seriously ill; one had attacks of ventricular fibrillation before pacing was started. Autopsy revealed large myocardial infarctions including both the anterior and posterior part of the septum in two and the antero-septal area in the other three.

In four patients ventricular fibrillation was directly provoked by the pacemaker equipment: one patient had ventricular fibrillation when the electrode was manipulated during positioning; two had an attack of ventricular fibrillation when accidentally paced by two pacemakers during measurement of threshold value shortly after the electrode had been positioned; and one patient had repeated short bouts of ventricular fibrillation during the first hours of pacing when the pacemaker impulse fell at the end of the T waves. These four patients survived.

Survivals

Thirty three patients (55 %) left the hospital alive. In at least five of these patients the symptoms directly related to the A-V block were very severe and were promptly relieved by the pacing. We believe the cardiac pacing saved the lives of these patients.

Only one patient was discharged with a permanent pacemaker. This patient had an anterior myocardial infarction and bundle branch block.

Deaths

Of the 60 patients with A-V block 27 (45 %) died. Two patients died during positioning of the electrode before cardiac pacing was established. They were both extremely ill, unconscious and in shock.

Table IV *Indications for pacing other than A-V block*

Indication	No. pts	Deaths
Cardiac standstill	9	8
S-A block	1	0
Nodal bradycardia	1	1
Sinus bradycardia	2	0
Atrial tachycardia with block	1	1
Ventricular tachycardia	1	1
Ventricular fibrillation	2	2

Nineteen patients died during pacemaker therapy. Twelve of them were in an extremely bad condition before the therapy was started with hypotension and cardiogenic shock. The ultimate cause of death was ventricular fibrillation in five, cardiogenic shock in ten, congestive failure in three and myocardial rupture in one.

Six patients died after pacing had been stopped; four of them in congestive failure. Before equipment for constant cardiac monitoring was available in the hospital, one patient in whom pacing had been stopped after a few days was found dead. After this episode we have as a rule kept the pacing catheter in place for three weeks and we have never stopped pacing during this period unless the patient was under continuous monitoring, thus making it possible to resume pacing immediately if necessary. One old demented patient pulled out the electrode and pacing was not resumed. He died suddenly after 18 days.

Autopsy

Autopsy was performed in 25 of the 27 fatal cases. In 13 the myocardial infarction was extensive, affecting approximately one third or more of the left ventricle. In the remaining 12 the extension of the myocardial infarction was moderate.

Isolated posterior myocardial infarction was only seen in four of these 25 cases. All of them died in cardiogenic shock.

In two cases the electrode was found in the coronary sinus. Perforation of the heart by the electrode was not found in any case.

Prognostic factors

Of 31 patients with isolated posterior myocardial infarction, four died (13 %) of 13 with isolated

anterior infarction nine died (69%). Ten patients with anterior infarction in combination with other locations all died.

Of seven patients with A-V block of second degree only one died while of 53 patients with complete A-V block 26 died (49%).

Of 31 patients who had bundle branch block 21 (68%) died (Table II). Among 29 patients in whom bundle branch block was not observed six (21%) died. Among patients with ECG evidence of isolated posterior myocardial infarction and bundle branch block mortality was 17% against 12% in patients with narrow QRS complexes.

Mortality related to the indication for pacing appears from Table II. The mortality was far higher in patients who had Adams-Stokes attack (61%) than in patients on prophylactic pacing (30%).

In patients with total A-V block the mortality was 41% when the A-V block appeared within 24 hours after onset of the myocardial infarction and 62% in patients who developed A-V block later. In half the number of patients with anterior myocardial infarction the A-V block developed late and the prognosis in these patients was extremely poor (Table III).

Indication for pacing on indications other than A-V block

In 17 patients with acute myocardial infarction endocardial pacing was started on indications other than A-V block. These patients who form a heterogeneous group will be mentioned only briefly. In nine of the patients the indication for pacing was cardiac standstill i.e. circulatory arrest with no atrial or ventricular activity in the electrocardiogram. This occurred after defibrillation for ventricular fibrillation in 4 patients. The other indications appear from Table IV. Thirteen of these patients (76%) died.

Ventricular fibrillation during pacing was seen in three of the patients. In one patient with sinus bradycardia an attack of ventricular fibrillation was triggered when the pacemaker was started. Pacing was stopped, the patient defibrillated and survived. Two other patients died after attacks of ventricular fibrillation. In one of them the indication for pacing was recurrent attacks of ventricular fibrillation, the other was paced after nodal bradycardia. All these patients were on fixed rate pacing.

DISCUSSION

The high mortality in patients with acute myocardial infarction and A-V block will in many cases be directly related to the conduction disturbance. On the other hand many of these patients have a severe coronary artery disease with extensive myocardial infarctions. A large proportion of the fatalities therefore are inevitable and due to extensive destruction of the myocardium. But as the present report has shown, cardiac pacing in acute myocardial infarction obviously may save some patients.

In this material as in others dealing with patients on pacemaker therapy mortality was greatest in patients with anterior myocardial infarction probably due to the great extension of the infarction (4). In patients with isolated posterior myocardial infarction the prognosis is far better. Lammers and Julian found a mortality of 27% in patients with posterior myocardial infarction and 91% in patients with anterior infarction (6). Patients with isolated posterior myocardial infarction and bundle branch block have a higher mortality than patients with narrow QRS complexes. This is in accordance with observations made by others (6). Patients with anterior myocardial infarction usually have bundle branch block when the A-V block appears.

Patients with acute myocardial infarction are more liable to develop ventricular fibrillation than other patients. In paced patients this complication may be provoked by mechanical or electrical stimulation from the electrode. The risk of causing ventricular fibrillation therefore has to be weighed against the positive results obtained by the treatment of the heart block. Normally the threshold for provoking ventricular fibrillation is 10-30 times the threshold for ventricular stimulation (11). Myocardial ischemia will lower the fibrillation threshold considerably as demonstrated in experiments on dogs by Wijffers et al. (13). A patient with acute myocardial infarction is therefore probably more liable to develop a pacemaker induced ventricular fibrillation than other patients. Ventricular fibrillation is most liable to occur in patients with competition between sinus rhythm and pacemaker induced rhythm because many pacemaker impulses will then fall in the vulnerable phase at the end of the T wave. Therefore patients with acute myocardial infarction should preferably be paced by

a ventricular inhibited on demand pacemaker. Such pacemakers are now available commercially.

In some cases it has been observed that the amplitude of the intracardial potential picked up by the pacing electrode is too low to inhibit the demand pacemaker which will then work at a fixed rate (12). If pacing has to be done at a fixed rate the lowest possible pacing stimulus should be used. The pacing threshold is altered by eating, sleeping and by many drugs; variations up to 30% are observed (9, 11). If fixed rate pacing has to be used in acute myocardial infarction the pacing stimulus should therefore probably exceed the threshold value by 30–50%. During the first days the pacing threshold will normally increase and the stimulus has to be adjusted accordingly.

We have used the soft and flexible Elema unipolar pacing catheter. These catheters are sometimes difficult to position. To position the catheter the patient invariably has to be turned on the left side. A stiffer catheter or a catheter with a guiding stylette is easier to insert especially if TV monitoring is available and turning of the patient is not necessary. With a stiff catheter however there is a risk of perforating the right ventricle. Therefore as a routine method to be used by physicians without previous experience in cardiac catheterization a soft catheter is preferable. Positioning of catheters during TV monitoring is the method of choice but insertion guided by intracardiac ECG can safely be used when TV monitoring is not available.

The alternative to electrical stimulation is pharmacological therapy. In acute A–V block the infusion of isoprenaline is usually effective also in patients with myocardial infarction. We have used isoprenaline infusion before placement of the pacemaker in our patients but we have not relied on this as the only therapy. Disadvantages of isoprenaline in these patients are the tendency to provoke ectopic tachyarrhythmias and the forceful drive on the heart with oxygen wasting effect in the myocardium.

The real reduction in mortality which is achieved by endocardial pacing in A–V block is difficult to assess. In 67 patients with advanced heart block Cohen reported a mortality of 75% in anterior myocardial infarction and 37% in diaphragmatic myocardial infarction (2). Average mortality in 17 reported series of patients with

advanced heart block was 58% (10). Mortality rates in hitherto published reports on paced patients are slightly lower but comparison is difficult due to selection of patients (6, 8, 10, 12).

In our opinion cardiac pacing ought to be instituted in patients with anterior myocardial infarction and advanced second degree or complete A–V block. The prognosis in this patient group is poor due to the usually very extensive myocardial infarction. However an Adams Stokes attack or extreme bradycardia due to A–V block most likely will make the condition even worse. Some authors also advocate prophylactic endocardial pacing in patients with anterior myocardial infarction and left bundle branch block as these patients are liable to develop a sudden total A–V block (7).

In patients with posterior myocardial infarction and narrow QRS complexes the prognosis is good. We have however observed Adams Stokes attacks in such patients and we therefore believe that endocardial pacing ought to be instituted when complete A–V block occurs even if the patient has a satisfactory heart rate. The natural history of the second degree A–V block in patients with posterior myocardial infarction and narrow QRS complexes is at the present time not known. Patients with posterior myocardial infarction and second degree A–V block with Wenckebach's periods might be kept under observation without pacemaker in a coronary care unit with facilities for emergency therapy.

We believe that the results achieved by endocardial pacing in acute myocardial infarction will be improved by the use of on demand pacemakers in order to avoid pacemaker induced ventricular fibrillation by reducing the pacing stimulus to the lowest possible level if fixed rate pacing has to be used and in patients with advanced A–V block by prophylactic pacing to avoid complications such as Adams Stokes attacks or severe bradycardia.

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A CASE OF KLINEFELTER'S SYNDROME WITH 48,XXXY AND DIABETES MELLITUS

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Abstract The twentieth case of Klinefelter's syndrome with 48,XXXY in a 39 year-old Caucasian is described. The outstanding clinical feature was a diabetes of the maturity onset type of nine years duration but with a somewhat slow plasma insulin response and an early peak of plasma growth hormone.

The patient was slightly dysphonic and immature with no sexual libido or potency and with a total IQ of 74. During a six months course of testosterone his general condition improved and alterations in his mental and sexual condition were also noted.

Cells from buccal smears and fibroblasts from the testis and the skin cultures were sex chromatin positive with a considerable number of double positive nuclei. In 209 analysed metaphases 78 had the modal chromosome number of 48 with a karyotype of 48,XXXY whereas 72 were non modal cells, preferentially with a hypomodal chromosome number. Analysis of metaphases with one missing chromosome indicated random loss of chromosomes except in cells from testis biopsy where the lacking chromosome belonged to the group 21-22-Y which makes a testis mosaicism possible.

PATIENTS WITH KLINEFELTER'S SYNDROME 48,XXXY

Nineteen cases of Klinefelter's syndrome with 48,XXXY have previously been described four from Canada by Barr et al (1) and Carr et al (5) two from USA by Ferguson Smith et al (8) and later by Money and Hirsch (25) and five from Great Britain by MacLean et al (19) and Hunter (15). Three such patients have been described in Japan one by Makino et al (20) and a pair of monozygotic twins by Takayasu et al (31). One patient with 48,XXXY has been described in France by Gilly et al (12) two cases from Sweden by Lambert (14) and two from Denmark by Nielsen and Fischer (28) and Frø-

land (11). IQ was below 70 in all patients but the one found by Nielsen and Fischer in 1965 had an IQ of 72. All patients were typical cases of Klinefelter's syndrome. One was described as schizophrenic and one suffered from a psychogenic psychosis.

Mosaics with 47,XXY/48,XXXY have been described in two mentally retarded patients one from Canada by Barr et al (2) and one from USA by Finley et al (9).

DIABETES MELLITUS IN PATIENTS WITH KLINEFELTER'S SYNDROME

Single cases with Klinefelter's syndrome and diabetes mellitus have been published by Lange (18), Castleman and Kibbee (6), Yodaiken (36), Benda et al (4), Fraser (10), Rhode (30), Zaninowich (37) and Lamotte (17).

Becker et al (3) found mild diabetes mellitus in five out of 50 patients with Klinefelter's syndrome giving a frequency of 10%. Jackson et al (16) studied the glucose metabolism in seven patients with chromatin positive Klinefelter's syndrome and one patient with chromatin negative Klinefelter's syndrome. Only one of the eight patients had a family disposition to diabetes mellitus; his paternal grandfather had diabetes. One of the eight patients had a diabetic glucose tolerance curve and one an increased early insulin response. Menzinger et al (21) found normal glucose tolerance curves in eight patients with Klinefelter's syndrome.

Mirouze et al (24) studied the glucose metabolism in four patients with Klinefelter's syndrome and found prediabetic insulin glucose

tolerance values in all four patients Nielsen (27) found that four of ten patients with Klinefelter's syndrome for whom a glucose tolerance test was available had blood sugar of 120 mg/100 ml or higher at two-and a half hours using the Hagedorn-Norman-Jensen method of glucose determination giving a frequency of 40%. Two fathers and three mothers among the parents of 25 patients with Klinefelter's syndrome suffered from diabetes mellitus (10%). Diabetes mellitus was found in the relatives of eight of the 25 patients with Klinefelter's syndrome (32%).

Wais and Salvati (34) found three patients with diabetes mellitus out of 32 patients with Klinefelter's syndrome giving a frequency of nine per cent. Four fathers and three mothers of the 32 patients had diabetes mellitus (11%). Diabetes mellitus was found in the near relatives in eight of the 32 patients (25%).

Zuppinger et al (38) found a diabetic glucose tolerance curve in six of 24 patients with Klinefelter's syndrome giving a frequency of 25%. Two of the 24 patients had clinical diabetes mellitus. Three fathers and one mother of the 24 patients suffered from diabetes mellitus (8%), and diabetes mellitus was found among near relatives of 11 of the 24 patients (46%).

Nielsen et al (29) found diabetic glucose tolerance in 12 of 31 patients with chromatin positive Klinefelter's syndrome (39%). All three patients with the chromosome constitution 48,XXXY including the patient presented in the present paper had a diabetic glucose tolerance.

HUMAN GROWTH HORMONE IN PATIENTS

WITH KLINEFELTER'S SYNDROME

Glick et al (13) found low fasting serum growth hormone levels in two patients with Klinefelter's syndrome. Nielsen et al (29) found a normal mean fasting plasma growth hormone level in 26 patients with chromatin positive Klinefelter's syndrome but 35% had an early plasma growth hormone peak compared to only 8% in a control group.

CASE REPORT

Medical Evaluation

A 39-year-old male who during the preceding ten years had suffered from several attacks of thrombophlebitis

was admitted to the hospital (1965/66) because of severe thrombophlebitis of the right leg with several chronic inflamed ulcers on the crus and foot. There was no family history of diabetes but in 1957 at the age of 30 an oral glucose tolerance test was found diabetic. The blood sugars at that time were: fasting 110 mg, 15 min 212 mg, 30 min 248 mg, 45 min 257 mg, 60 min 244 mg, 90 min 216 mg, 120 min 175 mg and 150 min 130 mg all per 100 ml. No dietary treatment or insulin was given.

On admission the physical examination revealed an eunuchoidal 173 cm tall prematurely aged man with fine wrinkles around the eyes and mouth. His weight was 105 kg. The facial hair was absent and the pubic and axillary hair was scant. The penis was small and the prostate not palpable. The right testis was absent. In the left scrotum a small soft structure was felt which in connection with biopsy through an inguinal incision was seen mainly to consist of a normal epididymis. The testis proper measured 8 × 10 × 20 mm. Histological examination revealed only fibrous tissue with a small island of non-characteristic epithelial tissue which might be Leydig cells. Hyalinized seminiferous tubules were not present in the biopsy specimen.

On the right foot and the right lower crus were several ulcers from pin size to 40 × 20 mm. There was pronounced edema of the region and the skin was dry, reddish discoloured and finely desquamating. The temperature was occasionally slightly elevated (38–38.5) during the first week after admission.

The remaining physical examination was normal. In particular funduscopy revealed no late diabetic manifestations.

Examination of the blood showed a sideropenic anemia with Hb 9.7 g/100 ml, Se-Fe 26 µg/100 ml and Se-transferrin 361 µg/100 ml. There were normal electrolytes and no acidosis. Electrophoresis of serum showed a slight reduction of the albumin fraction. The urinary sediment was normal and Se-creatinine 0.9 mg/100 ml. X-rays of the lungs and heart and an electrocardiogram were normal. X-rays of the bowel and stomach revealed a large esophageal hiatus hernia which was held responsible for a detected slight bleeding. An X-ray of the skull was normal. The electroencephalogram was slightly abnormal with some 5–7 c/s predominantly over the left hemisphere.

Basal metabolic rate, protein bound iodine, triiodo-L-thyronine absorption test and Se-cholesterol were normal. Se-cortisol showed a normal diurnal variation and ACTH and metopirone tests were normal. Pituitary gonadotropin excretion was increased (70–265–220 mouse units/24 h) and Se-testosterone decreased (0.2 µg/100 ml). The oral glucose tolerance curve (Fig. 1) was diabetic and the fasting morning blood sugar around 250 mg/100 ml. There was severe glucosuria but no ketone bodies in the urine. The response of plasma insulin during the glucose load was slight corresponding to a moderately severe glucose intolerance and the serum growth hormone showed an early peak at two hours (The assays of plasma insulin and growth hormone were done by Drs K. Johansen and H. Ørskov, Second University Department of Internal Medicine, Aarhus Kommunehospital).

Blood coagulation was slightly abnormal. The thrombo-

plasma activation test and coagulation time were short ended, while the plasminogen concentration was excessively high (355.2 μ g Cu Tyrosin/ml). The remaining coagulation tests, the number of thrombocytes, the coagel retraction, the recalcification and partial thromboplastin tests, the prothrombin (Quick) the prothrombin proconvertin and factor V tests, the plasma thrombin test, fibrinogen concentration, euglobulin test and thromboplastin generation test were all within normal limits.

Psychiatric Evaluation

The father, a smallholder, was 39 years old when the patient was born. He suffered from asthma and died after a brain hemorrhage at the age of 75. The mother was 30 years old when the patient was born; she is alive and healthy. The patient is number three of four siblings; he has two brothers and one sister. One brother suffers from asthma and the other brother has a tumor on the scrotum. The sister has two healthy children, but four of her children were either stillborn or died shortly after birth; two of them were twins.

The patient grew up in a good and harmonious home. He was very much tied to his mother and he was the most spoiled of the four siblings. He never participated in boys' play or sports of any type. At school he was not doing as well as his siblings; he had quite a lot of trouble with arithmetic and writing, but he got along quite well with the teachers as well as his schoolmates. He left school after the compulsory seven years, and he has stayed at home with his parents since then. He has received disablement pension since the age of 35. He and his mother have some home work together: packing bandages for the local hospital.

The patient never had any erection or pollutions and he never masturbated. He has not developed any sexual libido and he never felt like having and never had a girl friend or sexual relations.

In the medical ward he behaved very childishly but was happy, satisfied, friendly and joking. He got along quite well with the other patients and he was not teased.

At the psychiatric examination he appeared immature, quiet, passive and slightly apathetic, but he willingly answered the questions. He was somewhat dysphonic but not depressed. There were no psychotic and no neurotic symptoms.

Psychological testing with WAIS showed a verbal IQ of 74, a performance IQ of 76 and a total IQ of 74, with a comparatively great variation in the results from subtest to subtest. There was a pronounced tendency to self-centered, concrete thinking, and his imagination was colored by childish needs.

The personality tests also revealed primitive and oral content in fantasies, and there were further signs of identification difficulties. The childish primitive way of adaptation was supported by defence mechanisms, such as denial and repression, and his conflicts were brought out by questions related to identification.

Course and Treatment

The patient's diabetes was regulated with diet and 36 international units of long acting insulin upon which the

blood sugar stabilized within three weeks after admission at 80-270 mg/100 ml during the day and the urine was without or showed only a trace of sugar. The patient furthermore received oral administration of iron and diuretics and his ulcers were treated conservatively.

After two weeks of observation the patient experienced a new attack of thrombophlebitis of the right leg and developed an enormous edema of the right crus and foot which persisted together with the ulcers. Five weeks after admission the patient suddenly developed fever (39.9 °C) an infection with staphylococcus aureus was established and quickly controlled by combined treatment with penicillin, methicillin and fusidic acid.

Two months after admission the hemoglobin concentration started to increase but did not reach normal range until after nine months of oral iron medication.

Four weeks after admission treatment with 50 mg every third week of a testosteroneisobutyrate preparation with protracted action was started and continued for six months. Clinically the patient responded to this treatment by developing greater initiative and his general strength and condition improved. Within one month an increase in the length of the penis was noticeable and the patient began to experience erections several times a day especially stimulated by the sight of women on TV and he also started to masturbate and experienced a few ejaculations.

Within a week after the start of testosterone treatment the edema of the right leg decreased and the ulcers improved. Unfortunately the staphylococcal infection came at this moment and the above mentioned antibiotic treatment was given for seven days. After one month only one ulcer over the right medial malleolus remained which however persisted during the observation period of eight months. The attacks of thrombophlebitis terminated.

The blood coagulation tests were repeated and were normal one and six months after the initiation of the testosterone treatment, but remained normal two months after the termination of the treatment.

A psychiatric evaluation after five months of testosterone treatment revealed that the patient was more open and talkative and that dysphonic tendencies were no longer apparent. The patient had felt the development of sexual libido and masturbation disturbing. Because the creation of sexual libido with no potency at the age of 39 in such an immature man may be quite dangerous, leading to pedophilia, the testosterone treatment was definitely withheld.

Chromosome Analysis

(The cytogenetic part was done by
G. Bruun Petersen.)

Material and methods: Buccal smears for sex chromatin investigation were stained by the Feulgen method using hydrolysis in 5 N HCl at room temperature for 1 h followed by staining for 2 h.

Leucocytes from peripheral blood were cultured using the method of Moorhead et al. (26). Two samples of peripheral blood and two skin biopsy specimens were taken with an interval of six months. Skin fibroblasts were grown and later prepared for chromosome analysis.

Table I Number of cells counted and analysed in various tissues

Tissue	Experiment no	Chromosome count								Total no of cells analysed
		44	45	46	47	48	49	50	51	
Blood culture 1	PB 265	2		7	14	36		1		60
Blood culture 2	PB 271			1	2	27	1			31
Skin culture 1	H 183			1		28	1			30
Skin culture 2	H 189					30				30
Left testis	T 13	3	1	4	6	42	1		1	58
Total		5	1	13	22	163	3	1	1	209

by the technique described by Therkelsen (32) as were also fibroblasts from the left testis

Results Buccal smears were sex chromatin positive as 40 of the nuclei contained one and 13 of the nuclei contained two sex chromatin bodies (200 nuclei were examined). Cultures from skin as well as testis biopsies were also sex chromatin positive with a considerable number of double positive nuclei.

A growth curve experiment performed with the skin fibroblast cultures has been published earlier (33). It was shown that the frequency of double positive cells rose to close on 100% in the postlogarithmic growth phase. Of the 1006 cells examined two cells had three and four cells had four sex chromatin bodies.

Chromosomes were counted and analysed in 109 metaphases. The results are given in Table I. The modal chromosome number was 48 in all the cultures. In all modal cells (78%) the karyotype was consistent with the 48,XXXXY complement. An example of the karyotype is shown in Fig. 2.

Of the aneuploid metaphase five showed a hypermodal chromosome number. Two of these had the karyotype 49,XXXXY,C+ but one had in addition D trisomy and F monosomy. One metaphase had 49,XXXXY,C+ plus a

fragment. One had 48,XXXXY plus a fragment and the metaphase with 51 chromosomes had several abnormalities.

The existence of hypomodal stem lines in blood cultures made possible by the great number of hypomodal cells in this tissue is unlikely when the results in Table II which give the analysis of 12 metaphases with the chromosome number $2n=47$ are considered. In cultures from the testis biopsy, however, all the missing chromosomes belonged to the G group (21-22,-Y). Furthermore the analysis of all the hypomodal metaphases in the testis culture showed that among a total of 79 lacking chromosomes 13 were from the G group. Estimating the 99% confidence limits for the frequency P of lacking group G chromosomes from our results of 13 lacking G group chromosomes out of a total of 29 we get $0.001 < P < 0.69$. As the P value expected is 5.48 ± 0.10 , our results seem to indicate a significant departure from random loss of chromosomes in testis culture.

DISCUSSION

Generally the clinical manifestations were not different from those presented by patients with Klinefelter's syndrome 47,XXY and a high excretion of pituitary gonadotropin and low serum testosterone were found. All patients with Klinefelter's syndrome and 48,XXXXY have been mentally retarded with IQ below 75, whereas patients with Klinefelter's syndrome and 47,XXY have a mean IQ around 90. Our patient had a full scale IQ of 74 and he was immature and passive with no sexual libido or potency.

The finding of 39% with diabetic glucose tolerance among patients with Klinefelter's syndrome by Nielsen et al (29) indicates that the chromosome constitution 47,XXY and 48,XXXXY predispose to chemical diabetes mellitus and there is most probably a relation between the diabetes mellitus in the present patient and his chromosome constitution of 48,XXXXY.

The diabetes mellitus of the present patient was

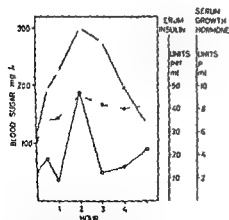


Fig. 1 Serum insulin and growth hormone response during an oral glucose tolerance test. x—x Blood sugar ●—● serum insulin ○—○ serum growth hormone

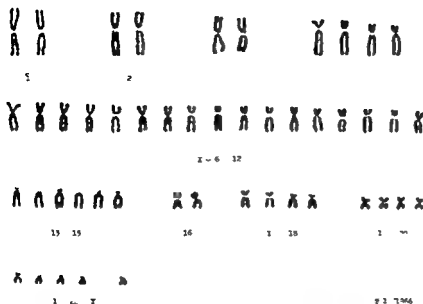


Fig. The karyotype from a cell with the 48,XXYY complement.

detected at the age of 30 by an abnormal glucose tolerance curve and the disease ran its course during the following nine years without giving rise to symptoms of glucosuria even when the patient on several occasions was hospitalized with thrombophlebitis. In connection with the present admission high blood sugar and severe glucosuria were present without however giving rise to ketonuria or acidosis in spite of a slight fever. The patient had not recognized any diabetic symptoms. Clinically the diabetes thus appeared to be of the maturity onset type but the patient's response of plasma insulin upon a glucose load was not so delayed and pronounced as is usually seen in maturity onset diabetes. The observed early peak in plasma growth hormone is more characteristic of diabetics of the juvenile type (35) but a similar early peak has been observed

in 35% of 26 patients with Klinefelter's syndrome (29).

The existence of a slight coagulation abnormality possibly a decreased fibrinolytic activity in a case of XXXY syndrome with nine years of recurring attacks of thrombophlebitis could suggest a causal relationship to the chromosomal abnormality. The other two known XXXY patients in this country were therefore investigated but showed completely normal coagulation tests and the coincidence therefore appeared fortuitous.

The evaluation of the effect of testosterone treatment on the ulcers of the leg is difficult. The ulcers appeared to improve in conjunction with the start of the treatment but they may also have improved because the diabetes had been brought under control even if the patient at the start of the testosterone treatment had received

Table II Results of analysis of 22 metaphases with one lacking chromosome ($2n - 47$)

	Number of cells lacking a chromosome in group						Total
	X-6-12		21--Y		Other		
	Observed	Expected	Observed	Expected	Observed	Expected	
Blood cultures	6	5.7	2	1.7	8	8.7	16
Left testis	0	2.1	6	0.6	0	3.3	6

Expected values were calculated on the assumption that all chromosomes have an equal chance of getting lost.

insulin for one month without showing any apparent improvement in the ulcers and even experienced a new attack of thrombophlebitis. Also the patient received antibiotic treatment for a short period soon after the institution of the testosterone treatment which may have promoted the tendency of healing. The coagulation abnormality disappeared upon treatment with testosterone but did not reappear after withdrawal of the treatment. It is thus not justified to assume a connection between the testosterone treatment and the disappearance of the minimal coagulation abnormality and the improvement in the leg ulcers.

The cytogenetic investigation showed that the patient has a modal chromosome number of 48 in all three tissues examined and in all 163 metaphases with the modal chromosome number the karyotype was 48,XXX.

The frequency of non modal cells was 22%, but the analysis of non modal cells in blood cultures showed random loss of chromosomes. A stem line with fewer than three X chromosomes is excluded in skin cultures by the fact that the frequency of cells with two sex chromatin bodies rose to very close on 100% in the post logarithmic growth phase. A stem line with more than three X chromosomes is unlikely on account of the few cells with more than two sex chromatin bodies.

In testis however all the 2n=47 metaphases had a lacking chromosome in group G. Further more among a total of 29 lost chromosomes in this tissue there was a significant departure from random loss in the G group ($P < 0.01$) and the possibility of mosaicism in testis must be taken into account. Miller et al (23) have shown that the Y chromosome has a peripheral localisation in the metaphase plate just like the 21-22 autosomes (22). Court Brown et al (7) showed that in males past 65 years the incidence of XO cells increases with age. This may give a greater chance of loss of the Y chromosome or of a chromosome in the 21-22 members. But as in blood cultures (Table II) there is a good agreement between observed and expected numbers we favor testis mosaicism as the most likely explanation.

The question of whether the lacking chromosome is a Y chromosome or a 21-22 autosome cannot be resolved as the patient's Y chromosome shows no identifiable characteristics (Fig 2).

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STUDIES IN URINARY TRACT INFECTIONS

I The Diagnosis of Bacteriuria in Women

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Abstract: Bacterial counts of successively collected mid stream urines and of urine obtained by suprapubic needle aspiration are compared. Bacterial counts in 79% of the patients with bacteriuria showed fewer than 10^4 organisms per ml. It is concluded that with regard to the number of organisms, no definite boundary exists between contamination and counts indicating bacteriuria with sufficient certainty.

More than 10^4 bacteria per ml in two consecutive mid stream specimens or more than 10^4 in a single specimen indicates bacteriuria. If fewer than 10^4 organisms per ml are found in a single concentrated clean voided urine specimen bacteriuria may be excluded. Bacterial counts in the range of 10^2 to 10^4 in a single mid-stream specimen from a woman do not permit a diagnosis of bacteriuria with sufficient certainty.

Demonstration of bacteriuria i.e. a state in which active growth is occurring in the urinary passage (2) is essential for the diagnosis of urinary tract infection. The introduction of quantitative urine culture in clinical practice (5, 8, 12) has largely made it possible to distinguish between bacteriuria and contamination in clean voided urine specimens. The use of percutaneous needle aspiration of the bladder (9) makes it possible to collect specimens aseptically. This permits the diagnosis of bacteriuria regardless of the number of organisms found.

The term "significant bacteriuria" is generally used when bacterial counts in clean voided specimens indicate bacteriuria with a degree of certainty sufficient for clinical practice. Too little attention has often been paid to the dependence of the criteria for significant bacteriuria upon the persons examined, the methods of urine collection and the number of specimens cultured.

In the present study bacterial counts on successively collected mid stream specimens and on

urine aspirated by suprapubic puncture have been compared.

MATERIAL AND METHODS

Over a period of eighteen months bacterial counts were made on urine specimens obtained from 458 non pregnant women between the ages of 16 and 65. These women were referred to the out patient clinic of the Roskilde County Hospital because of symptoms of urinary tract infection. One urine specimen was obtained from each patient on each of two consecutive days. In 717 cases a third specimen was examined.

Before the collection of the urine the patients were carefully instructed by a nurse who washed the vulva with sterile water using sterile cotton wool swabs. The last of these was left in the vagina. The labia were held apart by the patient, and approximately half way through the micturition a sterile plastic cup was introduced into the stream of urine (14). The urine was decanted into sterile tubes and immediately stored at 4°C.

During the last twelve months of the study suprapubic bladder puncture was attempted at the second visit of all new patients who had not urinated during the last two hours before arrival. The puncture was performed with the patient in the supine position. The skin was disinfected with iodine and the puncture was made in the midline about 3 cm above the symphysis pubis using an 8 cm long, needle no. 8 (intramuscular needle) attached to a 10 ml syringe. The needle was passed through the abdominal wall and directed toward the pelvic cavity. No local anesthetic was used. 6 to 8 ml of urine were aspirated from each of 95 women.

Quantitative bacterial culture of the urine was performed using the calibrated loop technique on nutrient agar and on blood agar plates and by streaking of the inoculum with a sterile glass rod (4). The plates were examined after 24 and 48 hours at 35°C.

RESULTS

A comparison of the bacterial counts of mid stream urine specimens obtained from the same

Table I Comparison of bacterial counts of two consecutive mid stream urine specimens from 458 women

First specimen Organisms/ ml	No of pats	Second specimen Organisms/ml				
		< 10 ³ ()	10 ³ - 10 ⁴ ()	10 ⁴ - 10 ⁵ ()	10 ⁵ - 10 ⁶ ()	> 10 ⁶ ()
< 10 ³	93	47	43	4	6	0
10 ³ -10 ⁴	121	41	47	4	4	3
10 ⁴ -10 ⁵	38	16	18	32	13	21
10 ⁵ -10 ⁶	55	11	13	14	26	36
> 10 ⁶	151	0	3	2	16	79

patient at different times showed that the count of a second and a third specimen agreed with the first count if fewer than 10⁴ or more than 10⁶ organisms per ml were found in the first specimen. However in four of 42 women with fewer than 10⁴ in two consecutive mid-stream specimens bacteriuria was demonstrated by culture of urine aspirated by suprapubic puncture.

The probability of error in accepting results in the range of 10⁴ to 10⁶ organisms per ml in a single specimen was found to be substantial (Table I) but the demonstration of 10 to 10⁶

two consecutive clean voided specimens brings the agreement with a third specimen up to approximately 90% (Table II). In all of 29 patients with more than 10 in two consecutive mid stream specimens bacteriuria was demonstrated in urine obtained by suprapubic puncture.

In 46 patients the aspirated urine was sterile. In two cases mid stream urine collected in connection with the puncture contained more than 10 organisms per ml (Table III) but in neither case did the bacterial count agree with the count of another mid stream specimen from the same patient.

Table II Per cent of successively collected mid stream specimens containing more than 10⁵ or fewer than 10⁴ bacteria per ml in relation to the number of bacteria found in the first specimen (I) and in the first two specimens with concordant counts (II)

No. of bacteria per ml in first specimen (I) and in the first two specimens (II)						Total no of specimens examined
	< 10 ³	10 ³ -10 ⁴	10 ⁴ -10 ⁵	10 ⁵ -10 ⁶	10 ⁶	
Per cent of specimens with more than 10 ⁵ organisms/ml	I 6 II 0	7 6	34 71	67 89	95 93	458 217
Per cent of specimens with fewer than 10 ⁴ organisms/ml	I 90 II 100	88 94	34 29	24 11	3 3	458 117

Table III Bacterial counts in urine obtained by suprapubic needle aspiration and in mid stream urine specimens collected in connection with the puncture

Organisms/ ml in the mid stream specimens	Organisms per ml urine aspirated by suprapubic puncture					
	Sterile	< 10 ³	10 ³ - 10 ⁴	10 ⁴ - 10 ⁵	10 ⁵ - 10 ⁶	> 10 ⁶
< 10 ³	27	1	1	0	0	0
10 ³ -10 ⁴	17	1	5	0	0	0
10 ⁴ -10 ⁵	0	0	2	3	1	0
10 ⁵ -10 ⁶	0	0	0	1	0	1
10 ⁶	0	0	0	0	1	26
Total no of pats	46	2	8	4	8	27

Even though none of the patients was heavily hydrated or had received any antibacterial agent prior to the needle aspiration fewer than 10 organisms per ml were found in 29% (14/49) of the patients with bacteriuria. From 12 of the 14 patients with fewer than 10 in the aspirated urine another mid stream specimen collected during a urine concentration test after admission to the hospital contained more than 10. The two remaining patients probably attained sterile urine spontaneously.

DISCUSSION

In patients with urinary tract infection a considerable multiplication of bacteria takes place in the urine in the urinary tract (5, 13). The number of bacteria in clean voided specimens from these patients usually far exceeds the number of bacteria due to contamination from the urethra and the perineal region (6, 12, 14). In clinical practice quantitative urine culture is therefore used to

distinguish between bacteriuria and contamination

In the majority of their patients with symptoms and signs of urinary tract infection Marple (8) and Kass (6) found more than 10 organisms per ml in catheter and clean voided urine specimens. Stamey et al (13) found fewer than 10 in 33% of the specimens aspirated by suprapubic puncture from patients with bacteriuria and Goldberg et al (3) found fewer than 10 in 45% of the specimens in a similar study. In both of these studies the puncture was performed after a period of heavy hydration which may have caused a considerable decrease of the bacterial count (3, 11). In the present study needle aspiration was done without preceding hydration and fewer than 10 organisms per ml were found in 29 of the patients with bacteriuria.

Several authors have found that the number of bacteria in clean voided specimens from women with no symptoms of urinary tract infection rarely exceeds 10^4 per ml (6, 12, 14). In a study involving 54 women whose urine was sterile when obtained by needle aspiration Stamey et al (13) found however that mid stream specimens collected in connection with the puncture contained 10^4 to 10^6 bacteria per ml in 11% and more than 10 in 7% of these cases. Beard et al (1) found that mid stream specimens from 68 puerperal patients with sterile urine contained more than 10^4 in 12 cases. In the present study more than 10 bacteria per ml were found in 4% of the mid stream specimens collected from 46 women whose urine was sterile.

It must therefore be concluded that with regard to the number of organisms no definite boundary exists between contamination and significant bacteriuria in women. The problem is to find out whether the results of quantitative urine cultures of one or more clean voided specimens indicate the presence or absence of bacteriuria with a degree of certainty sufficient for clinical practice.

By comparing two bacterial counts of urine obtained from the same patient at different times Kass (7) found that the second specimen contained more than 10^3 in less than 2% of the cases when the number of bacteria in the first specimen was below 10^4 . In the present study culture of successive specimens revealed bacteriuria in 3 of the women with fewer than 10^4 in the first

specimen. Bacteriuria was however demonstrated by culture of urine obtained by needle aspiration in 4 of 42 women with fewer than 10^4 organisms per ml in two consecutive mid stream specimens. In most of the cases of bacteriuria with low bacterial counts a third count on a concentrated urine specimen exceeded 10^4 .

In clinical practice bacteriuria may therefore be excluded if a single concentrated clean voided specimen contains fewer than 10^4 bacteria per ml.

Kass (7) found that when the bacterial count of a clean voided specimen was in the range of 10^3 to 10^4 per ml a second specimen contained more than 10 organisms (Gram negative rods) per ml in only 67% of the cases. In the present investigation 62% of such patients had more than 10 in a second specimen. It must be stressed that 10^3 to 10^4 in a single clean voided specimen from a woman do not indicate bacteriuria with sufficient certainty.

The presence of 10 or more bacteria per ml in two consecutive mid stream specimens or more than 10 in a single specimen increases the probability of bacteriuria to approximately 95%.

Discrepancy between two bacterial counts or counts in the range of 10^4 to 10^6 does not permit any conclusion. Either the examination should be repeated on a concentrated urine specimen or suprapubic needle aspiration should be performed.

However it must be kept in mind that the diagnosis of bacteriuria based on the results of quantitative cultures of clean voided specimens rests upon the application of statistical probabilities to individual urinary findings (10).

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STUDIES IN URINARY TRACT INFECTIONS

II Urinary Tract Infection Due to Coagulase negative Staphylococci

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Abstract Coagulase negative staphylococci were found in the urine of 14% of 193 non pregnant women between the ages of 16 and 65 with significant bacteriuria. The leucocyte excretion rate in the urine of these patients was increased and was found to be of the same magnitude as in cases of infection due to bacteria known to be pathogenic in the urinary tract. Pyuria disappeared as the patients obtained sterile urine. It is concluded that the patients were infected with coagulase negative staphylococci in the urinary tract.

The majority of the patients had a depression of renal concentration capacity. There was good agreement between the concentration capacity and clinical symptoms normally regarded as indicative of pyelonephritis. It seems probable that coagulase negative staphylococci may cause pyelonephritis.

Coagulase negative staphylococci were more frequently found in younger patients with urinary tract infection and were more frequent among patients without previous symptoms than among those with a history of recurrent urinary tract infection.

In comparison with patients with bacteriuria with *E. coli* patients with coagulase negative staphylococci obtained spontaneously sterile urine more frequently and more rapidly.

Within the last few years coagulase negative staphylococci have been demonstrated in urine aspirated by suprapubic bladder puncture (14, 21, 23) in urine obtained from the ureter (3) and the renal pelvis (18) and by the culturing of renal biopsies (1, 18).

It is often considered questionable whether coagulase negative staphylococci are pathogenic in the urinary tract. A study has therefore been undertaken of the symptoms and the results of urine and renal examination of women with bacteriuria with coagulase negative staphylococci.

MATERIAL

Since October 1966 general practitioners in the Roskilde area have been invited to refer all non pregnant women

between the ages of 16 and 65 with symptoms or signs of urinary tract infection to the outpatient clinic of Roskilde County Hospital.

Most of the patients have been referred because of acute symptoms of urinary tract infection others have come due to unexplained symptoms such as tiredness, backache or headache. A few patients with asymptomatic urinary tract infection have been referred by doctors because routine examination revealed pyuria, hematuria or proteinuria.

As of April 1968 416 women had been examined at the hospital. Significant bacteriuria was found in 193 patients, all of these were admitted to the medical department for further investigation.

METHODS

All the women were interviewed by the author and came to the hospital on two consecutive days for urine examination. The specimens were collected by a practised nurse using the mid stream technique described by Vejlsøgaard (5). From February 1967 suprapubic bladder puncture was attempted on all new patients who had not urinated during the last two hours before arrival.

Quantitative bacterial culture of the urine was performed using the calibrated loop technique on nutrient agar with lactose on blood agar plates, and by streaking of the inoculum with a sterile glass rod (6). Bacteriuria was diagnosed by isolation of bacteria from urine obtained by suprapubic puncture or demonstration of significant bacteriuria, i.e. more than 10^5 bacteria in two consecutive mid stream specimens (7, 13).

Identification of the isolated bacteria was undertaken by the Department of Diagnostic Bacteriology, Statens Serum Institut, Copenhagen. Microchemical characteristics of some of the isolated coagulase negative staphylococci have been published by Mortensen (17).

A method for determination of the leucocyte excretion rate in urine has previously been described (17) and the excretion in normal persons has been found to be less than 400 000 leucocytes per hour. With the technique employed for microscopic examination of urinary sediment it has been shown that three or more leucocytes per HPF indicate pyuria (1). Hematuria was diagnosed by demonstrating three or more erythrocytes per HPF or by definite positive benzidine reaction in the urine.

The renal concentration capacity was estimated by

with signif

either 0.5 g of sulphamethazole or 0.75 g of ampicillin for 14 days. A third of the patients were treated with placebo using the double blind technique. The tablets were distributed in numbered bottles, every third bottle containing placebo tablets.

RESULTS

Of the 193 non pregnant women between the ages of 16 and 65 with significant bacteriuria 27 had bacteriuria with coagulase negative staphylococci (Table I). Twenty two of these had more than 10^4 organisms per ml in at least two consecutive urine specimens. Two had more than 10^7 in one specimen and more than 10^6 in the other and one had between 10^6 and 10^7 in both specimens. In the remaining two cases where there were fewer than 10^4 bacteria per ml the diagnosis was confirmed by culture of urine aspirated by suprapubic puncture. One additional patient was found to have a mixed infection with E. coli and coagulase negative staphylococci. The diagnosis was confirmed by bladder puncture; this patient is not however included in the material under consideration here.

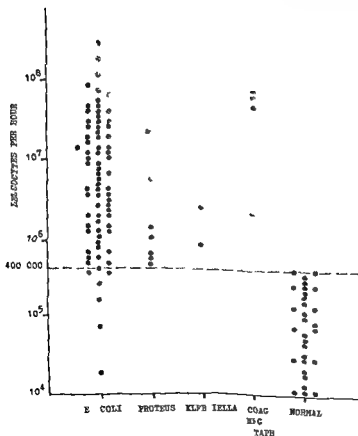


Fig 1 Hourly leucocyte excretion in the urine in 32 normal women and in 177 women with bacteriuria. The latter group is subdivided according to organism isolated.

The leucocyte excretion rate for the last 20 patients was determined after admission to hospital. The leucocyte excretion was found to be of the same magnitude as in infections due to bacteria known to be pathogenic in the urinary tract (Fig. 1).

A comparison of the results of microscopy of urine sediment in the presence of bacteriuria and the findings 14 days after the urine had become sterile was made to determine further the correlation between pyuria and bacteriuria with coagulase negative staphylococci. Of the 27 patients examined 22 had three or more leucocytes per HPF in the first specimen and 26 in at least one specimen before the urine became sterile. No patient had more than two leucocytes per HPF after two weeks with sterile urine.

Among all patients 34% had hematuria and 47% had urinary protein concentrations exceeding 0.25 ppm. Forty-four per cent of the patients with coagulase negative staphylococci had hematuria and more than 0.25 ppm of protein was found in 44% of specimens from these patients.

Urinary tract infection due to coagulase negative staphylococci was most frequent among the younger patients (Table II). Twenty-three % of the patients between 16 and 25 were infected with coagulase negative staphylococci while only 8.5% of the patients over 25 had coagulase negative staphylococci in the urine ($\chi^2=7.670$, $p<0.01$).

All the patients have been under continued observation and by April 1968 there had been 130 instances of significant bacteriuria after a period with sterile urine, 4.6% of which were reinfections with coagulase negative staphylococci. Urinary tract infections due to coagulase negative staphylococci were found to be considerably less frequent among the reinfected patients than in the original material of 193 patients ($\chi^2=7.48$, $p<0.01$).

Coagulase negative staphylococci were found more frequently in patients without previous symptoms of urinary tract infection than in patients with previous symptoms (Table III). No patients with asymptomatic urinary tract infection had coagulase negative staphylococci in the urine. A relatively large number of the patients with coagulase negative staphylococci had backache and three patients had clinically acute pyelonephritis with fever and flank pain (Table IV).

Table II Age distribution

Age y	16-5	26-35	36-45	46-55	56-65
All patients	39	20	19	9	13
Patients with coag. neg. staph	63	7	7	11	11

Table III Previous symptoms

I No previous symptoms of urinary tract infection II One to three episodes of dysuria and frequency III More than three episodes of dysuria and frequency IV Previous symptoms of pyelonephritis with fever and flank pain associated with symptoms from the lower urinary tract

	I	II	III	IV
All patients	38	26	19	27
Patients with coag. neg. staph	44	22	15	19

Table IV Subjective symptoms upon arrival at the hospital

I Dysuria and frequency II Dysuria, frequency and backache III Backache only IV Flank pain and fever V No subjective symptoms

	I	II	III	IV	V
All patients	48	21	5	11	15
Patients with coag. neg. staph	48	41	0	11	0

Table V Duration of symptoms before first examination

Duration (days)	1-3	4-10	11-30	>30	No symptoms
All patients	27	29	24	8	15
Patients with coag. neg. staph	37	33	27	4	0

The duration of symptoms before arrival at the clinic was approximately the same among patients with coagulase negative staphylococci and in the material as a whole (Table V).

The serum creatinine concentration did not exceed 1.1 mg per 100 ml for any of the patients. Intravenous urography was performed on all patients; one patient showed slight signs of pyelonephritis.

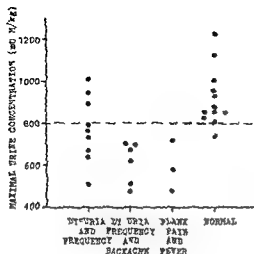


Fig. 2 Maximal urine concentration capacity in relation to clinical symptoms in 20 patients with urinary tract infection due to coagulase negative staphylococci. The figure shows in addition the concentration capacity in 15 convalescents without signs or symptoms of urinary tract disease.

phritis and one patient had a dilatation of one ureter with no indication of obstruction. No definitely abnormal condition was discovered in any of the other patients.

The maximum urine concentration capacity as determined for the last 20 patients. The results are shown in Fig. 2 together with the concentration capacity in 15 convalescents without present or previous symptoms of urinary tract disease. Seventeen of the patients with urinary tract infection due to coagulase negative staphylococci had a maximum urine concentration capacity of less than 800 mOsm per kg. There was good correlation between symptoms normally considered indicative of pyelonephritis and the concentration capacity (Fig. 2). Some correlation was found between subjective symptoms and protein concentration in the urine (Table VI).

In five cases the serum was tested for antibodies against the isolated coagulase negative staphylococci upon arrival at the clinic and 14 days later. A definite rise in titre was found in only one case. An inhibition test showed that the antibodies were neutralized by the strain of coagulase negative staphylococci isolated from the patient. However, this patient had no other signs of acute pyelonephritis and other patients with clinically acute pyelonephritis had no antibody titre rise.

Table VI Symptoms and urinary protein concentration in patients with coagulase negative staphylococcal bacteriuria

I Dysuria and frequency II Dysuria, frequency and backache III Flank pain and fever

	I	II	III
Protein concentration			
Less than 1/4 %	9	6	0
1/4 % to 1 %	4	5	1
1 % or more	11	0	2

All patients treated with sulfamethizole or ampicillin had sterile urine within one week after the start of treatment. After completion of treatment one patient relapsed with coagulase negative staphylococci after a period with sterile urine.

A third of the patients, nine in all, were treated with placebo. In the course of two months eight of these obtained sterile urine spontaneously. One patient had bacteriuria with coagulase negative staphylococci for a period of eight months without any subjective symptoms. At this time she became pregnant and was treated. In all the placebo-treated patients who had bacteriuria for more than one week the subjective symptoms disappeared before the urine became sterile. The proportion of patients with sterile urine in relation to all the placebo-treated patients during the observation period is shown in Fig. 3 together with the course of 31 placebo-treated patients with bacteriuria with *E. coli*.

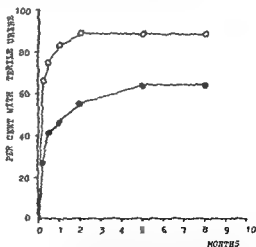


Fig. 3 Per cent of placebo-treated patients with sterile urine in the observation period. O—O patients with coagulase negative staphylococci. ●—● patients with *E. coli* bacteriuria.

DISCUSSION

Coagulase negative staphylococci are usually considered harmless saprophytes. Through investigation of the bacterial flora in the female urethra Cox (2) found staphylococcus albus to be the organism most frequently isolated. This probably explains the fact that this is a frequent contaminant in specimens obtained via the urethra (11, 22). The demonstration of coagulase negative staphylococci in the urine has therefore been regarded with scepticism.

Within the last few years several authors (14, 21, 23) have found coagulase negative staphylococci in urine aspirated by suprapubic bladder puncture. Coagulase negative staphylococci have also been found in urine obtained from the ureter (3) and from the renal pelvis (18) and by culture from renal biopsies (1, 18).

It is often considered questionable whether coagulase negative staphylococci are pathogenic in the urinary tract. The author therefore examined 27 women with significant bacteriuria with coagulase negative staphylococci for other signs and symptoms of urinary tract infection.

Pereira (20), Gallagher et al (5) and Mitchell (16) state that most of their patients with coagulase negative staphylococci in the urine had pyuria as determined by microscopy of urine sediment.

In the present study the leucocyte excretion rate was determined and found to be of the same magnitude as in infections due to bacteria known to be pathogenic in the urinary tract. This confirmed that the patients had inflammatory disorders of the urinary tract (4). Judging by the results of examinations of urine sediment the pyuria disappeared in all patients within two weeks after the urine had become sterile. It must therefore be concluded that the patients were infected with coagulase negative staphylococci in the urinary tract.

The concentration capacity was reduced in most patients in the present study, and there was good agreement between subjective symptoms of pyelonephritis and depression of renal concentration capacity. Low concentration capacity does not in all cases however indicate a diagnosis of acute pyelonephritis. Some patients may have reduced concentration capacity due to irreversible chronic pyelonephritis. The patients are therefore under continued observation for possible changes in renal concentration capacity (26). On the basis

of the information available it must be assumed that coagulase negative staphylococci may cause pyelonephritis.

The serum of some patients was examined for antibodies against the isolated strains of coagulase negative staphylococci. This test was found to be of no value in the differential diagnosis between pyelonephritis and infection of the lower urinary tract.

Many authors have rejected coagulase negative staphylococci found in the urine regarding them as contaminants or as apathogenic. This makes it difficult to obtain an impression of the frequency of urinary tract infection due to coagulase negative staphylococci.

Through systematic investigation in a general practice of all patients with symptoms of urinary tract infection Gallagher et al (5) found 88 patients with significant bacteriuria. Fourteen of them were infected with coagulase negative staphylococci. In a similar investigation Steensberg et al (24) found significant bacteriuria in 223 mostly younger women. Coagulase negative staphylococci were isolated from 14% of the total number. In the same investigation bacteriuria was found in 34 males, only one of them had coagulase negative staphylococci in the urine.

McFayden and Ekyen (14) performed suprapubic bladder puncture on 1000 pregnant women and found 59 with bacteriuria. 11 of these had coagulase negative staphylococci. In a screening study of pregnant women Kincaid Smith (8) found coagulase negative staphylococci in 14 of the patients with significant bacteriuria while Little (10) in a similar investigation found this organism in only 1% of all patients with urinary tract infection.

In a general medical department Kleeman et al (9) found coagulase negative staphylococci in 1 of males and pregnant and non pregnant women with significant bacteriuria. Mitchell (16) reported that coagulase negative staphylococci were frequently found in bacteriuria following instrumentation of the urinary tract.

Among 193 non pregnant women between the age of 16 and 65 with significant bacteriuria coagulase negative staphylococci were found in 14, predominantly among the younger patients. It must be stressed that all of them were patients who normally would have been treated by their family doctors and not referred to hospital.

All the patients are under continued observation so far 130 reinfections have occurred in only 4.8% of these reinfections have coagulase-negative staphylococci been found. This suggests that coagulase-negative staphylococci occur less frequently in patients who have recurrent urinary tract infection. This is in accordance with the fact that bacteriuria with coagulase-negative staphylococci is less common among hospital patients with chronic pyelonephritis who have been admitted because of recurrent or complicated urinary tract infection. The patients who had recurrences during the observation period cannot be compared without reservation with the original patient material because they differ from them in respect of age distribution and frequency of previous urinary infection.

In comparison with the rest of the patients those with coagulase-negative staphylococci had fewer previous periods with symptoms of urinary tract infection. However this relationship is of little significance because there is some correlation between age and frequency of previous infection.

Asymptomatic urinary tract infection may be so scantily represented among the patients studied as most of them sought their family doctors because of symptoms of urinary tract infection. However it must be noted that among 27 patients with asymptomatic bacteriuria there were none with coagulase-negative staphylococci in the urine.

A comparison of the duration of symptoms before the first examination at the hospital of patients with coagulase-negative staphylococci in the urine and the duration of symptoms among all the patients showed no differences. There was no indication that the first group sought a doctor sooner or later than the other. The discomfort suffered by both groups is apparently equally pronounced.

All the patients treated with chemotherapeutics or antibiotics obtained sterile urine within a week. Nine patients were treated with placebo and eight of these attained spontaneously sterile urine in the course of two months. Compared with the course of patients infected with *E. coli* patients with coagulase-negative staphylococci attained sterile urine more frequently and more rapidly. The groups are not however entirely comparable with regard to age and frequency of previous

urinary tract infection as selection was based on the type of organism isolated from the urine.

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STUDIES IN URINARY TRACT INFECTIONS

III Biochemical Characteristics of Coagulase negative Staphylococci Associated with Urinary Tract Infections

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Abstract Forty-six strains of coagulase negative staphylococci from 13 patients with established urinary tract infections have been examined by biochemical tests. None of the strains produced coagulase or fermented mannitol. The strains differed in their production of haemolysins and phosphatase, phage sensitivity, liquefaction of gelatin, reduction of nitrate, splitting of Tween 80, pigmentation and production of acid from glucose, mannitol and lactose.

It is concluded that contrary to prevailing opinion a negative result in the coagulase test on staphylococci isolated from urine does not exclude their association with infections of the urinary tract.

Although generally disregarded as agents of disease, coagulase negative staphylococci have been isolated from urine under circumstances which point to their role as pathogens by Pereira (14), Gallagher et al. (4), Roberts (15), Mitchell (9), Mabeck (8) and others.

Roberts (15) and Mitchell (9) identified the staphylococci isolated from the urinary tract by use of the key devised by Baird Parker (1).

The present paper deals with the biochemical characteristics of 46 strains of coagulase negative staphylococci isolated from 13 female patients with urinary infections.

MATERIAL AND METHODS

Forty-six strains of coagulase negative staphylococci from 13 patients with urinary infections were available for examination. The strains were isolated by Mabeck during the last part of his study when the possible pathogenic role of coagulase negative staphylococci in infections of the urinary tract was recognized. The association of these strains with urinary infection is based upon the criteria described by Mabeck (7), all of which were fulfilled. All 46 strains were isolated on different occasions from

mid stream urine and in some cases, from urine obtained by suprapubic aspiration of the bladder.

Morphology, Gram staining, motility, arginine decomposition, production of catalase, coagulase, deoxyribonuclease, oxidase, phosphatase and urease (method b only), gelatin hydrolysis, H₂S formation, reduction of nitrate, splitting of Tween 80 and the Voges-Proskauer reaction were performed and read all according to the methods described by Mortensen and Kocur (10).

Production of acid from carbohydrates: pH of cultures grown aerobically and anaerobically in a fluid medium containing glucose was determined as described by Mortensen and Kocur (10). The semisolid Standard Medium of the Subcommittee on Taxonomy of Staphylococci and Micrococci (10) and the medium of Hugh and Leifson (5) were used with and without a paraffin oil cover for measurement of production of acid from glucose and mannitol. The Hugh and Leifson medium was used without a top layer of paraffin oil for the determination of acid production from arabinose, galactose, maltose and lactose.

Production of β -galactosidase was examined by use of an o-nitrophenyl β -D-galactoside (ONPG) test in the modification of Bulow (3).

Gelatin liquefaction was also studied by Kohn's method as modified by Lautrop (6); a positive result by either method is recorded as positive.

Haemolytic activity was assessed by placing a loopful of a 5-6 hour broth culture on nutrient agar plates with 5% defibrinated rabbit and sheep blood and subsequently incubating at 35°C for 18 hours and then at 4°C for 24 hours.

Novobiocin sensitivity was examined by the prediffusion method of Thomsen (21) using a medium of beef heart broth containing agar approximately 18%, defibrinated horse blood, 10% glucose, 1% sodium chloride, 0.3% Na₂HPO₄, 12% H₂O. Paper discs impregnated with 25 μ g of sodium novobiocin (Antibiotic Dep. Statens Seruminstitut) were applied to the plates 18 hours before inoculation. By this method an inhibition zone with a diameter of more than 25 mm corresponds to a 0.0 inhibitory concentration of less than 2 μ g/ml and indicates sensitivity. The novobiocin sensitivity test

Table I *Production of acid from glucose in different media*

	Type of fermentation	pH in fluid medium		Standard medium		Hugh & Lefson medium	
		Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
A		4.3-4.8	4.3-4.6	+	+	+	+
B		5.2-5.8	4.9-5.3			+	+
Inoculated control tubes without glucose		6.8-7.2	6.9-7.9	-	-	-	-
Uninoculated tubes with and without glucose		6.8-7.0	6.9-7.2	-	-	-	-

+ = positive - = negative - = weak reaction in the top part of the medium

Range of values after incubation for 2, 4 or 6 days

was also performed with 5 sensitive and 5 resistant strains on the above medium without addition of glucose.

Phage typing was done by the method of Blair and Williams (2) using the international basic set of typing phages supplemented as described by Rosendal et al. (16) and by Rosendal and Bulow (17). All strains were examined using both the routine test dilution (RTD) and the dilution RTD $\times 1000$.

RESULTS

Common characteristics

All strains were found to be non motile. Gram positive cocci growing in pairs and clusters. All strains gave positive results in the tests for production of catalase, urease and acid from galactose and maltose. They gave negative results in the tests for production of coagulase, deoxyribonuclease, H₂S oxidase and acid from arabinose. No strains fermented mannitol by the criterion and method of the Subcommittee (19, 20). In the Voges-Proskauer test only some of the strains gave positive results after an incubation period of two days, but all were positive when the test was done after incubation for five days. All strains produced a reaction in the arginine test when this was performed with suspensions of bacteria.

Differential characteristics

The strains could be divided into two types (A and B) according to differences in their production of acid from glucose. This was shown by the pH changes taking place in fluid medium and by the colour changes occurring in the Standard medium (Table I).

Type A was characterized by pronounced and rapid formation of acid both aerobically and anaerobically with pH values ranging from 4.3

to 4.8 from the second day of incubation and by positive results in the Standard medium by the criterion of the Subcommittee (19). Type B also produced acid from glucose under anaerobic and aerobic conditions, but did so less markedly. pH values of the anaerobic cultures were in the range 5.2 to 5.8. In the Standard medium negative results were recorded although colour changes to yellow were observed in the upper part of the medium. In the medium of Hugh and Lefson all strains of both types A and B gave positive results.

Table II shows a division of the strains into biotypes taking into account all differences observed. The results of phage typing are listed in Table III. The variations in phage susceptibility among the strains from patient IM are considered too small to indicate distinct populations. With two exceptions strains isolated from the same patient belonged to the same biotype. One strain from patient EN differed from three other strains from this patient in three properties *viz* pigmentation, phage sensitivity and ability to liquefy gelatin. The strains from patient MH belonged to two different biotypes but these were isolated at an interval of over six months.

Strains having glucose fermentation of type A differed from type B strains in several other respects. They gave positive results in the arginine reaction by the method of Møller (13), produced phosphatase, reduced nitrate, split Tween 80 and were sensitive to novobiocin but did not produce acid from mannitol in either medium. The strains having glucose fermentation of type B displayed various patterns of behaviour and differed in their production of acid from lactose, phage sen-

Table II Differences between the strains

Table II Differences between the strains																	
Pat	No of strains	Type of glucose ferment	Arginine reaction	Haem of sheep red cells	Nitrate to nitrite	Novo biocin sens	Phosphatase	Tween 80	Mannitol			Lactose		Pigm. nt	Gelatin	Phase typing	
									Stand medium	H & L medium	An & ac	H & L medium	Ac				ONPG
VC	3	A	+	+	+	S	+	+	-	-	+	+	-	-	+	NT	
NT	4																
UH	2																
BMA	1																
MN	3	A	+	-	+	S	+	+	-	+	+	+	-	Orange	+	NT	
IM	3	B	■	-	-	R	-	-	+	+	+	+	-	Orange	-	+	
EN	3	B	X	-	-	R	-	-	+	+	+	+	+	Orange	+	NT	
EN	1	B	X	-	-	R	-	-	+	+	+	+	+	-	+	NT	
SP	11	B	X	-	-	R	-	-	+	+	+	+	Orange	-	-	NT	
LC	2																
MH	3																
MH	3																
MH	3	B	X	-	-	R	-	-	+	+	+	+	-	Orange	+	NT	
MH	3	B	X	-	-	R	-	-	+	+	+	+	+	Orange	+	NT	
KH	3	B	X	-	-	R	-	-	+	+	+	+	+	Orange	+	NT	
LH	3	D	X	-	-	R	-	-	+	+	+	+	+	Cream	+	NT	
LH	3	B	X	-	-	R	-	-	+	+	+	+	-	-	-	NT	
KJ	2																

+ = positive = weak reaction in the top part of the medium - = negative X = positive in rapid test negative in growth test
 S = sensitive R = resistant An = anaerobically ac = aerobically NT = not typable

See Table III

Table III Results of phage typing

Pat	Strains	Phage type / pattern	
		RTD	RTD × 1000
EN	6148	NT	83A (B)
	6149	NT	83A (B)
	6177	NT	83A (B)
IM	6498	NT (77/85 + w)	3A/3C/55 6/47E/47/54/77/81/83A/85
	6499	77/85 +	52/52A/79/80 3A/3C/55/6 42E/47/53/54/75/77/81/83A/84/85/4*D 6557/
	6300	NT	3A/3C/55/6/47E/47/54/75/77/81/83A/85 +

RTD = routine test dilution NT = not typable

sitivity, pigmentation and ability to liquefy gelatin. Haemolytic activity was recorded only in some of the strains of type A.

The glucose content of the medium in the novobiocin sensitivity test did not influence the division of strains into sensitive or resistant types but the inhibition zone produced by the resistant strains decreased from 9–12 mm to <6 mm in the medium without glucose.

DISCUSSION

Roberts (15) isolated Gram positive, catalase positive cocci from suprapubic urine specimens in 20 patients. By Baird Parker's key 14 were *Micrococcus* and six *Staphylococcus* strains. Eleven of the *Micrococcus* strains were of subgroup 3, two of subgroup 2 and one of subgroup 7. All strains of the *Staphylococcus* group belonged to subgroup II. Mitchell (9) reported that strains likely to have caused infection belonged to Baird Parker's *Micrococcus* subgroup 3 in 39 cases which corresponded to about a quarter of all the cases he examined the remainder belonging to the *Staphylococcus* subgroups II (72 patients), IV (4 patients), V (21 patients) and VI (11 patients). Thirty eight of the 39 cases of infection with *Micrococcus* subgroup 3 had acquired their infection outside hospital.

The findings reported in this paper confirm the occurrence of coagulase negative staphylococci in patients from domiciliary practice with infection of the urinary tract and the existence of different levels of glucose fermentation capacity among such strains. The strains isolated from 8 of 13 patients could be identified as *Micrococcus* by use of the scheme of Baird Parker and by their

production of acetoin they could be placed in one of his subgroups 1, 2, 3 or 4. However differences in the composition of the media used to detect acid produced from carbohydrates do not permit a definitive subgrouping. The strains isolated from the remaining 5 of the 13 patients gave similar reactions to those of his *Staphylococcus* subgroup II.

The proportion of strains fermenting glucose rapidly under anaerobic conditions differs from the frequency reported earlier by Mortensen and Kocur (11) where 269 of 285 clinical isolates exhibited such type A fermentation. However no isolates from urine were included in that study (12).

The positive results of phage typing are in accordance with the results of Spink and Strong (18) who found 16 strains of coagulase negative staphylococci phage typable among 485 clinical isolates.

The present work emphasizes that the results of a coagulase test should not be used indiscriminately as an indication of the pathogenicity of staphylococcal agents of infections of the urinary tract. Whether this holds true also for strains associated with other infections remains to be investigated more fully.

Furthermore the observations here reported on strains having an established association with infection show that pathogenicity cannot be excluded by the properties in which the strains differ (e.g. production of phosphatase and haemolysins, sensitivity to novobiocin, liquefaction of gelatin, pigmentation, reduction of nitrate, splitting of Tween 80 and production of acid from glucose, mannitol and lactose).

Therefore before clinical conclusions are drawn

from a laboratory report of isolation from urine of staphylococci whether coagulase positive or negative account should be taken of such other factors as the symptoms of the patient the conditions of sampling the number of bacteria per ml urine the excretion of leucocytes etc

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HEMODYNAMIC EFFECTS OF LIDOCAINE

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Abstract Lidocaine is currently one of the most used drugs in ventricular arrhythmias, especially in acute myocardial infarction. Only few series subjected to hemodynamic studies have hitherto been reported. The author studied the hemodynamic changes following the intravenous injection of lidocaine to 12 cases with non ischemic heart disease. After a bolus dose of 50 or 100 mg an infusion was started with 1 or 2 mg/min for 20 minutes. No significant changes were found in mean values for heart rate, cardiac output, stroke volume, mean systemic and pulmonary artery pressures or peripheral arterial resistance. It was well tolerated in all cases, including one with total A-V block. The serum lidocaine concentration was determined in eight cases.

After some earlier animal experiments (3) the first successful conversion of ventricular fibrillation in man was performed in 1942 with the aid of procaine (1). Though effective procaine has a depressant effect on blood pressure (15). Lidocaine (lignocaine, Xylocaine®) was synthesized in Sweden in 1943. It has gained widespread use as a local anesthetic but has also a place in the treatment of status epilepticus.

Lidocaine was first tried in ventricular fibrillation in 1950 by Southworth & M (19). In that case countershock was effective only after the intracardial administration of lidocaine. However procaine had been given previously though apparently without effect. Further lidocaine was given in a preparation with adrenaline 1:100 000 and in small doses. Thus it seems difficult to ascribe the effect in this case to one drug.

Since 1953 a number of papers have appeared on the use of lidocaine in experimental animals. Since about 1959 it has gained increasing clinical use as an antiarrhythmic agent. During the last years lidocaine has in many coronary care units, including our own, been the method of choice for the treatment of ventricular ectopic beats.

Since only few studies have been reported concerning the hemodynamic effects of lidocaine the present work was started. The main question has been the effects on heart rate, cardiac output and systemic arterial pressure in adult cardiac cases with different degrees of disability.

MATERIAL

Twelve cases between 24 and 61 years were referred for routine diagnostic heart catheterization. Their diagnosis are listed in Table I. They were all in function classes II-III. Sinus rhythm was present in eight cases, atrial fibrillation in three and total A-V block in one case. All cases were compensated at the time of the investigation.

METHODS

Right heart catheterization was performed in all cases, with percutaneous insertion of a polythene catheter in the brachial artery. In two cases a transseptal left heart catheter was also inserted. Only local anesthesia was used. After insertion of the catheters the patient rested for 10 min. Cardiac output was then determined using Fick's direct method. Expired air was collected during 10 min and the oxygen consumption was determined by Haldane's method. Arterial and mixed venous blood gases were analyzed by a spectrophotometric method.

Lidocaine was then injected via the right heart catheter. A bolus dose of 50 or 100 mg was injected during 1 min, immediately followed by a drip of 1 or 2 mg/min for 10 min. During the last 10 min cardiac output was measured. Thus a total of 70-140 mg was given during 10 min. The serum concentration of lidocaine was determined in eight cases by means of gas chromatography. The samples were taken 15 min after the injection.

RESULTS

These are presented in Table I. Irrespective of the doses given there were no statistically significant differences in mean values for cardiac output, heart rate, stroke volume or systemic arterial pressure.

Table I Hemodynamic changes following lidocaine administration

Case no	Age (y)	Sex	Diagnosis	Rhythm	Lidoc dose	Lidoc conc. ($\mu\text{g/ml}$)	CO (l/min)		HR		SV (ml)		Systemic arterial pressure (mm Hg)	
							B	A	B	A	B	A	B	A
1	53	♂	ASAI	A-V block	50+1	0.9	5.6	4.8	41	39	136	123	138/64	144/62
2	61	♀	Stat. p. myoc.	Sinus	100+2	1.5	4.3	4.6	60	61	72	76	133/80	140/81
3	24	♂	ASD op	Sinus	50+2	2.0	5.8	5.0	52	52	112	96	144/81	140/80
4	60	♂	MS+AI	Atr fibr	50+1	2.6	4.4	4.0	61	61	72	66	138/72	147/70
5	53	♀	Dolor pect	Sinus	50+1	5.2	4.4	4.9	53	54	81	91	132/80	135/80
6	50	♂	MS	Atr fibr	50+1	1.1	3.7	3.3	89	89	47	36	134/70	130/80
7	44	♂	MS	Sinus	50+1	—	3.6	3.7	71	70	50	53	125/70	125/70
8	44	♂	MSMI	Atr fibr	50+1	—	3.1	2.4	56	54	55	44	130/78	138/80
9	44	♂	Stat. p. myoc	Sinus	50+1	—	5.3	6.4	72	73	74	88	122/70	118/70
10	37	♀	ASD+MI	Sinus	50+1	—	10.7	17.3	75	76	271	276	121/68	107/70
11	41	♀	MS	Sinus	100+2	3.7	3.8	4.1	60	61	63	67	122/65	140/75
12	46	♂	MS	Sinus	100+1	6.5	3.7	3.9	57	57	63	68	120/67	123/71

AS = aortic stenosis. AI = aortic insufficiency. MS = mitral stenosis. ASD = atrial septal defect of secundum type. Stat. p. myoc = status post myocarditis. CO = cardiac output. HR = heart rate. SV = stroke volume. Pressures systolic/diastolic/mean. Case 10 CO and SV mean pulmonary blood flow and right ventricular stroke volume respectively.

Dose in mg and mg/min respectively. B = before, A = after lidocaine.

sure. This applies also to case 1 who had a third degree A-V block as well as to case 12 the only patient who experienced side-effects. Immediately after the bolus injection, she complained of slight headache which disappeared during the following constant infusion. The values for CO and SV in case 10 were not included in the statistical test. The values for oxygen consumption, arterial-venous oxygen difference, mean pulmonary artery pressure and peripheral arterial resistance are not reported. Nor were any statistically significant changes in these parameters found after lidocaine.

The mean percentual changes were for cardiac output -3% (range -23% to $+21\%$) for heart rate $\pm 0\%$ (range -5% to $+2\%$) for stroke volume -3% (range -20% to $+19\%$) for mean arterial pressure $+2\%$ (range -6% to $+19\%$).

In cases 8, 9 and 10 there were significant changes in cardiac output during xylocaine administration. No untoward effects were noted nor any restlessness or anxiety as regards the procedure.

Serum lidocaine concentrations varied between 0.9 and 6.5 $\mu\text{g/ml}$.

DISCUSSION

Experimental investigations have shown that, in doses comparable to those used clinically, lidocaine

has no or only a slight depressant effect on cardiac output (5, 14) though conflicting results have also been reported (2).

Only few series have hitherto been reported on its effects in patients. Kumacy and Steinhaus (15) and Harrison et al. (11) compared the effect of lidocaine with that of procaine and procainamide respectively in cases under general anesthesia. Recently some results have been published concerning the hemodynamic changes following lidocaine in patients without general anesthesia. In cardiac patients without myocardial infarction no consistent changes in cardiovascular performance were noted after a single intravenous injection of 50–100 mg (18) nor when a loading dose of 1 mg/kg was followed by 1 mg/min (4). However, though there were no significant changes in mean values, signs of a slight myocardial depressant effect was found in four out of eight cases who received a dose of 100 mg. These effects were of short duration and unaccompanied by any symptoms (18).

A few studies have been reported concerning the effects in cases with acute myocardial infarction (13, 20, 21). No significant changes were found in brachial artery pressures, heart rate, cardiac output or stroke volume after an intravenous injection of 1 mg/kg (13) or 100 mg (21).

These earlier results are confirmed by the present study in which a loading dose of 50–100 mg

was immediately followed by an infusion with 1-2 mg/min

The problems concerning dosage blood levels and toxicity have been analyzed (7 9 10 12 13). The therapeutic serum level is between 1 and 4 µg/ml. This is usually obtained with an infusion rate of 25-35 µg/kg/min which in a 70 kg man will correspond to 1.75-2.45 mg/min. Toxic effects have been noted at levels in excess of 6 µg/ml (6 10). As lidocaine is metabolized to a large extent in the liver great caution should be used when giving the drug to patients with impaired liver function. With higher serum concentrations there may be twitchings or general convulsions. It should be noted that immediately after a single injection of 1 mg/kg serum levels of about 10 µg/ml are recorded (13). This could explain the values found in the present study.

It has been stated that lidocaine is contraindicated in patients with A-V dissociation and a slow nodal or idioventricular pacemaker (8). In experiments on dogs with a surgically total A-V block lidocaine was found to slow the ventricular rate (7 16). In the case reported here (case 1) no untoward effects were noted after lidocaine. It has recently been found that lidocaine has no influence on the ventricular stimulation threshold in patients with total A-V block and pacemaker (17). However lidocaine should be used with great caution in cases with second and third degree A-V block unless a pacemaker is immediately available.

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NON HYPERTENSIVE ANGIOPATHY

Renal Biopsy Study of the Nephrotic Syndrome

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Abstract The thickness of the wall of the small arteries and arterioles has been measured microscopically from renal biopsies of 36 normotensive patients forming three groups: 1) 12 patients with lipid nephrosis or glomerulonephritis with the nephrotic syndrome, 2) 11 patients with glomerulonephritis without the nephrotic syndrome and 3) 13 patients with various unrelated diseases. Groups 2 and 3 served as controls. The patients with nephrosis showed thickening of the renal vessels. The difference from both control groups was statistically significant. The renin-angiotensin-aldosterone system operating in secondary hyperaldosteronism is thought to be the cause of the vascular changes.

Hypertensive angiopathy in familial chloride diarrhoea, a non-hypertensive state, was reported by Pasternack and Perheentupa (5). It was supposed that the vascular alterations (thickening of the walls of small arteries) were due to prolonged vasoconstriction caused by the high angiotensin activity related to the secondary hyperaldosteronism in this state. Recently Brackett et al. (3) reported a case of Bartter's syndrome having the same vascular alterations. Bartter's syndrome includes some of the characteristics of familial chloride diarrhoea, namely: juxtaglomerular hyperplasia, high renin activity, high aldosterone excretion and absence of hypertension.

Because of these observations we decided to study the arterioles and small arteries in kidney biopsies from non-hypertensive patients with the nephrotic syndrome, i.e. another state of secondary hyperaldosteronism.

MATERIAL AND METHODS

The series consists of renal biopsies from 36 patients with normal blood pressure and unimpaired renal function.

Twelve of the patients displayed the nephrotic syndrome. In four of them only minimal glomerular changes were noted in the renal biopsy and the aetiology was considered to be lipid nephrosis. In eight nephrotics the aetiology was chronic glomerulonephritis. All patients of this group were heavily oedematous at the time of the biopsy. Three of them had been treated with adrenocortical steroids maximally for three months.

Eleven patients with chronic glomerulonephritis without nephrosis were studied as controls.

Thirteen patients served as further control. In these patients the renal biopsy was done for various reasons irrelevant to the present issue. The morphological findings were normal.

The renal biopsy specimens were fixed in 10% neutral formaldehyde, embedded in paraffin wax, sectioned at 3-5 μ and stained with periodic acid-Schiff (PAS) and silver methenamine according to Gomori.

From each patient/biopsy specimen about ten cross sections of small arteries and arterioles were measured with an eye-piece micrometer under high power objective (400 \times). For each vascular cross section a quotient was calculated from the vascular and luminal diameter. The more thickened the vessel the greater the quotient.

For statistical analysis the mean quotient for each patient was calculated. The mean values of the nephrotic patients were statistically tested against the mean values of the patients of both control groups. Correction for small samples was performed (1).

RESULTS

The results are given in Figs 1-4. In Figs 1-3 the quotients of each vessel in the nephrotic and control groups are plotted in relation to the age of the patients. The figures show that there was a greater spread of the quotient values in the nephrotic patients than in the controls. In the nephrotic group there were 17/123 quotient values over 3.0 compared with 5/241 in the control series. In this series no correlation could be seen between the quotient values and the age of the

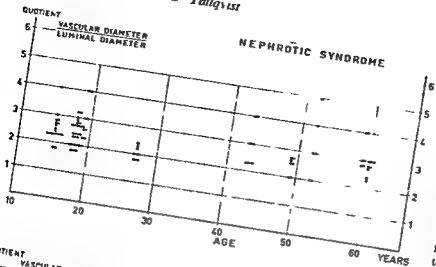


Fig 1 Quotient values (vascular diameter/luminal diameter) of small arteries and arterioles in relation to patient's age. Vessels from kidney biopsies of normotensive patients with nephrotic syndrome

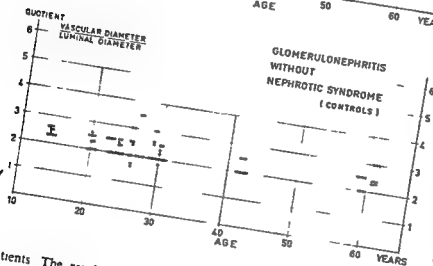


Fig 2 Quotient values (vascular diameter/luminal diameter) of small arteries and arterioles in relation to patient's age. Vessels from kidney biopsies of patients with glomerulonephritis without nephrotic syndrome and hypertension

The results imply that there are vessels of both normal and abnormal thickness in the nephrotic group

Fig 4 gives the mean quotient values of the patients in the different groups and the mean of each group. The difference of the mean values of the nephrotic group and the control groups is statistically significant (nephrotic syndrome/glo-

merulonephritis $P < 0.001$ nephrotic syndrome/other controls $0.01 > P > 0.001$)

The thickening of the vessels was mostly due to cellular hyperplasia. In a few cases the arteriolar wall showed strong PAS positive staining in some areas of the intima and media. The two changes occurred side by side in the same patients (Figs 5-7)

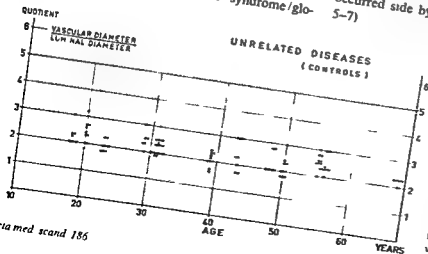


Fig 3 Quotient values (vascular diameter/luminal diameter) of small arteries and arterioles in relation to patient's age. Vessels from kidney biopsies of patients with unrelated diseases and absence of hypertension

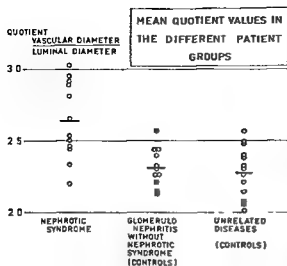


Fig 4 Mean quotient values of the patients in the different groups.

DISCUSSION

The method of measuring blood vessel thickness from histological sections is of course open to



Fig 5 Normal renal arteriole Silver stain, $\times 450$

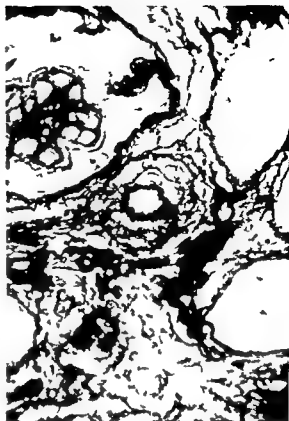


Fig 6 Arteriole with hyperplastic and numerous cells in the wall Kidney biopsy of a patient with nephrotic syndrome Silver stain, $\times 450$

criticism. The relatively sparse distribution of perpendicularly cut small arteries and arterioles necessitated the observation of several sections of the same sample. The risk of measuring the same vessel twice seems to be equal in the different patient groups. The fact that the tissue samples were of approximately the same size in all cases and that the process of fixing and embedding was standardized allows a comparison between the different patient groups.

From the results presented it becomes obvious that among the patients with glomerulonephritis there was a difference in the thickness of the small arteries and arterioles between those with and those without the nephrotic syndrome. The increased cellular hyperplasia of arteries and arterioles in the nephrotic group therefore seems to be somehow related to the nephrotic syndrome.

In the present series only three patients treated with corticosteroids were included. The vascular changes present in the nephrotic patients of this

Table II Coronary mortality and production of tobacco

	Mortality from myocardial infarctions per 100 000 inhabitants						Production of tobacco per 1000 inhabitants		
	♂			♀			Cigars (mill.)	Cigarettes (mill.)	Other tobacco products (kg)
	35-44	45-54	55-64	35-44	45-54	55-64			
Australia	66.6	312.1	874.9	15.3	75.7	304.0	0.0049	1.69	540
Austria	36.2	162.3	513.8	10.7	36.6	153.5	0.014	1.78	120
Belgium	37.4	141.0	407.2	8.5	33.1	115.3	0.029	1.44	860
Canada	70.3	304.1	814.5	12.0	58.7	272.3	0.018	2.07	580
Denmark	35.5	177.0	547.9	5.5	30.4	163.0	0.065	1.55	670
England	57.4	22.6	684.3	8.6	41.0	198.6	0.013	2.17	300
Finland	88.6	343.9	910.5	14.3	55.3	246.1	0.0038	1.27	170
France	19.4	72.6	200.8	3.5	13.1	60.8	0.0058	1.08	400
Greece	13.1	48.9	138.9	6.7	17.3	51.3	—	1.54	—
Israel	30.8	155.2	563.8	6.2	38.9	317.9	0.00081	1.15	19
Italy	33.2	128.8	387.4	10.8	40.4	165.2	0.072	1.08	97
Japan	18.5	52.0	151.8	16.7	35.1	95.9	0.00002	1.53	16
Netherlands	33.4	164.1	495.7	4.8	23.4	129.5	0.14	1.22	1020
New Zealand	54.8	273.3	771.6	11.8	56.1	268.0	—	1.32	850
Norway	38.4	169.5	526.1	4.4	24.3	149.1	0.0036	0.38	1220
Sweden	19.9	127.2	494.4	4.4	24.8	162.1	0.0017	0.90	500
Switzerland	33.8	130.2	445.5	8.3	33.9	167.3	0.18	2.05	320
USA	90.6	358.8	923.7	18.5	81.4	313.1	0.036	2.78	400
W. Germany	41.5	170.0	500.2	15.2	47.9	173.2	0.072	1.06	130
Yugoslavia	7.5	72.0	3.45	5.7	49.1	249.7	—	1.19	6

information was available on the consumption of various tobacco products the production figures were used as the point of comparison (Table II).

The results are shown in Table III. The only significant correlation established was between cigarette production and the male and female coronary mortality rates.

It is interesting that although the amount of coffee, tea, cocoa and alcohol consumed may be regarded at least to some extent as parameters of

the standard of living, no significant correlation was discernible between their consumption and the coronary mortality rate. On the other hand, there was a highly significant correlation between cigarette production and the coronary mortality rate, but no such correlation was demonstrable for other tobacco products. This correlation was equally significant with regard to both male and female coronary mortality, which is different from the standard of living parameters analysed earlier.

Table III Correlation coefficients between coronary mortality rate and coffee, cocoa, tea and alcohol consumption and tobacco production

	Coffee	Cocoa	Tea	Alcohol	Cigars	Cigarettes	Other tobacco products
♂							
35-44	0.090	0.19	0.32	-0.11	-0.12	0.61	0.11
45-54	0.14	-0.18	0.35	-0.17	-0.13	0.55	0.19
55-64	0.17	-0.086	0.40	-0.23	-0.11	0.52	0.18
♀							
35-44	-0.21	-0.13	0.15	-0.09*	0.15	0.54	-0.34
45-54	-0.098	-0.074	0.31	-0.23	-0.18	0.61*	-0.6
55-64	0.078	-0.060	0.38	-0.32	-0.18	0.49	-0.16

* $p < 0.05$ $p < 0.01$ * $p < 0.005$

for which the significant correlation was only with male mortality. Cigarette production is also correlated with the standard of living: the coefficient of correlation between e.g. the national income and cigarette production was 0.60 ($p < 0.005$).

It must be remembered in evaluating the results that the figures used are based on official statistics the reliability of which is not always very high. Especially the extent to which the official coronary mortality rate illustrates the prevalence of coronary heart disease can and has been criticised. It is clear in addition that the correlations established do not in themselves imply a causal relationship. Even with these reservations I think the correlations presented here and earlier are suggestive.

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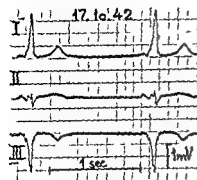


Fig 1 Case 24 Typical WPW

As to functional capacity the patients who were still alive in 1967 were grouped according to the criteria of the New York Heart Association.

The death certificates of the patients who had died were requested. Case records of patients who had died in a hospital were studied with special reference to cardiac state and autopsy findings. The patients who had died outside hospital had all been hospitalized within a few years of death and these records were also obtained for study. All patients seem to have known something about their cardiac disorder as in all case records great attention had been paid to the patient's cardiac state.

The expected number of patients alive in 1967 was calculated from tables of mortality rates in Denmark for five year periods for each age and sex.

RESULTS

1 The Material as a Whole

All 47 patients could be traced. In 1967 28 patients were still alive whereas 19 had died. Table 1 shows the age and sex distribution of the patients at the age of the first ECG shown, WPW and at the time of re-examination or death. It will be appreciated that about half of the

Table 1 Age and sex distribution of 47 patients with WPW syndrome

Age (y)	Number of patients		
	At first ECG	On re-exam	At death
0-9	1	0	0
10-19	2	0	0
20-29	23	0	2
30-39	6	0	2
40-49	6	8	2
50-59	4	13	3
60-69	2	3	2
70-79	3	4	5
80-89	0	0	3
Total	47	28	19
Sex	♂ 24 ♀ 23	♂ 12 ♀ 16	♂ 12 ♀ 7

Table 2 Duration of PR, QRS and delta waves in 46 patients with WPW syndrome

PR (sec)	No of pats	QRS (sec)	No of pats	Delta (sec)	No of pats
		0.10	3		
		0.11	4		
0.06	4	0.12	12		
0.07	3	0.13	7	0.03	3
0.08	17	0.14	11	0.04	14
0.09	7	0.15	5	0.05	5
0.10	9	0.16	2	0.06	16
0.11	3	0.17	1	0.07	7
0.12	4	0.18	1	0.08	2

patients were between 20 and 29 years of age when the first ECG showing WPW was recorded. The oldest patient was 75, whereas in one patient (no. 28) this pattern was present as early as 1924 when as a 7-year-old boy he was hospitalized because of rheumatic fever.

a Electrocardiographic findings

The diagnosis of WPW syndrome could be confirmed in all 47 cases. Table 2 summarizes the electrocardiographic characteristics relevant to this diagnosis. It will be appreciated that ten patients were borderline cases in respect of one of the electrocardiographic features considered characteristic of WPW. Thus PR was as much as 0.12 sec in four patients. All these patients had QRS complexes of 0.13 or 0.14 sec and delta waves of 0.04 sec or more. In three patients QRS was 0.10 sec, whereas PR values for the same patients were 0.08, 0.09 and 0.11 sec and conspicuous delta waves were present being 0.04 and 0.05 sec. In three patients the delta waves were only 0.03 sec but P-R intervals were 0.08 or 0.09 sec and QRS complexes 0.12 sec. Further contribution to the diagnosis was offered by the fact that in all ten patients an ECG recorded on another occasion showed definitely longer P-R intervals, narrower QRS complexes and absence of delta waves.

WPW was a concomitant finding in 20 patients who were investigated for non-cardiac diseases. The remaining 27 patients were referred because of cardiac symptoms.

One patient had only one ECG taken. In 34 patients five ECGs or more were available and in 16 cases 10 ECGs or more were recorded. In 25 of the 46 patients who had more than one ECG taken the WPW pattern was intermittent (Fig. 2) and in six patients alternation between WPW and normal complexes was seen in the same ECG (Fig. 3).

b Paroxysmal tachycardia

From the case records of the patients who had died it appeared that 11 had given a history of paroxysmal tachycardia, which was also reported by 18 of the patients still alive. Thus 29 patients (62%) probably had paroxysmal tachycardia. This was confirmed by an ECG in 11 patients. Ten of them had regular supraventricular tachycardia (Fig. 4) and one had atrial fibrillation (Fig. 5).

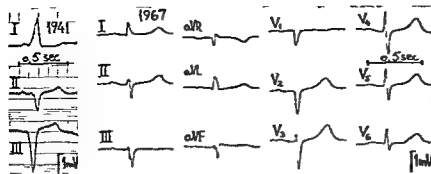


Fig 2 Case 12. 1941 typical WPW 1967 normal conduction.

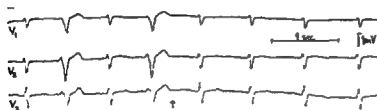


Fig 3 Case 4 Alternating normal complexes and WPW Normalization during breath holding (at arrow)

One patient had frequent extrasystoles and attacks of supraventricular tachycardia (Figs. 6 and 7)

Twenty-one patients had had attacks for more than one year and six patients for less than one year when the diagnosis of WPW was made. One patient had his first attack ten years later at the age of 49. In one patient it was not possible to find out exactly when the attacks had started and in another information about the duration of the attacks could not be obtained. Table III shows

the age distribution at the first attack of tachycardia, and Table IV the maximal duration of the attacks. It will be appreciated that 19 patients had experienced paroxysms of one hour's duration or more although usually the duration of the attacks was 10 min or less in 20 patients, and 15-30 min in three patients.

In eight patients the attacks were invariably followed by polyuria. Three patients had experienced one or two syncope related to tachycardia.

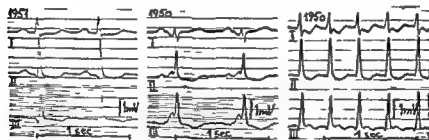


Fig 4 Case 11 1951 normal conduction 1950 WPW (center) and supraventricular tachycardia (right)

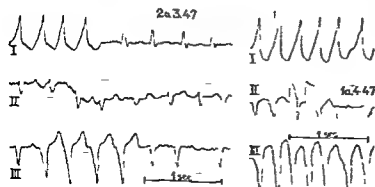


Fig 5 Case 11 Atrial fibrillation with runs of beats at a high rate and with the configurational characteristics of WPW complexes recorded in the same patient during sinus rhythm

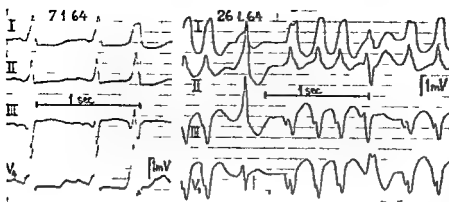


Fig 6 Case 35 WPW with single and serial extrasystoles.

As could be expected the frequency of attacks varied considerably from one patient to another and also in the same patient from time to time. In seven patients it could be stated that the frequency of attacks had decreased with advancing years, whereas it had increased in eight patients. Of two patients who are still alive one has had no attacks whatsoever since 1944 and another had his latest paroxysm in 1960.

II Findings on Re examination of Patients

Still Alive

1967 8 patients aged 44-77 years were still alive. Twenty six patients were re-examined and two answered a questionnaire. The observation period was 40 years for one patient (no 28) and varied between 23 and 34 years for the remaining 27 patients.

a Electrocardiographic findings

The ECG showed WPW in 12 patients. In one (Fig 3) WPW and normal complexes alternated in a bigeminal fashion, and WPW complexes normalized during Valsalva manoeuvre or breath holding. In another patient the P waves were abnormal, being negative in leads II, III and aVF pointing to retrograde atrial activation (Fig 8). QRS complexes were normal, RR intervals were 0.12 sec in leads III and aVF. On most other occasions her ECG had shown WPW (Fig 9). In still another a man aged 77 years coupled extrasystoles were periodically present (Fig 10). This finding had been reported in his case sheets as early as 1943.

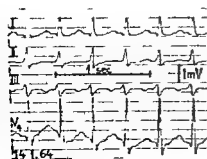


Fig 7 Case 35 Supraventricular tachycardia.

b Paroxysmal tachycardia

Eighteen patients had paroxysmal tachycardia. In 15 patients the usual duration of the attacks was 10 min or less. In three patients the attacks usually lasted 2-4 and 17-24 hours respectively. The maximal duration of the attacks was less than $\frac{1}{2}$ -hour in one patient and $\frac{1}{2}$ -1 hour in five patients. Three patients had had occasional attacks of 24 hours duration or more. The frequency of paroxysms varied considerably, some patients having several attacks a week and others only one or two attacks a year.

c Functional capacity

Eighteen patients reported no restriction of their functional capacity. Nine patients aged 48-73 years belonged to functional group II, their main symptoms being dyspnea on exertion and palpitation. Seven of them gave a history of paroxysmal tachycardia. One patient (no 4) received an invalidity pension because of these attacks. In two patients there were other than cardiac reasons why their functional capacity was restricted. One a man aged 53 had had pulmonary tuberculosis for which a thoracoplastic operation had been performed. He had never had paroxysmal tachycardia. Another a woman aged 50 (no 16) had chronic bronchitis, arterial hypertension and slight right and left heart failure and had frequent attacks of tachycardia. One patient was incapacitated by a paralysis of her calf muscles caused by a long standing lumbar nerve root compression.

d Associated findings

One patient, a man aged 67 (no 1) gave a history consistent with earlier myocardial infarction and reported

Table III Age at first paroxysm of tachycardia

Age (y)	No of pats
< 0	11
20-29	1
30-39	4
45	1

Table IV Maximal duration of tachycardia

Duration (h)	No of pats
< $\frac{1}{2}$	4
$\geq \frac{1}{2}$ < 1	5
> 1 < 24	13
≥ 24	5

anginal pain at rest as well as on exertion. His ECG at rest in the absence of WPW did not show ischemic changes. Another man aged 49 years (no 47) had had angina on exertion for two years. His ECG at rest showed WPW but no definite signs of ischemia. The above mentioned woman aged 50 (no 16) had typical angina on exertion. Again the ECG at rest showed WPW but no unequivocal signs of myocardial ischemia. As mentioned she also had arterial hypertension.

Arterial hypertension was found in two other women: one aged 51 (no 6) the other aged 66 (no 13). Patient no 6 probably had more widespread affection of the arterial system as she had rough murmurs over the carotid arteries and the aorta and inequality of carotid brachial and radial pulses.

Cardiac murmurs were heard in five patients. The above mentioned woman (no 6) had a rough grade 2 systolic murmur at the apex and over the aorta. A 47-year-old man (no 28) who had had rheumatic fever had an accentuated first sound at the apex and a grade 2 mesodiastolic rumble with presystolic accentuation findings consistent with a mitral stenosis. Three other patients had grade 1-2 systolic murmurs in the second and third left intercostal space.

Chest roentgenograms displayed normal cardiac silhouettes in most of the patients. Only one had a slightly enlarged heart (no 4) the volume being 560 ml/m.

c Cardiac medication

Only three patients received constant cardiac medication. All three received digitalis because of paroxysmal tachycardia (nos 4, 6, 16). A thiazide diuretic was given to two patients with arterial hypertension (nos. 6 and 16) and nitroglycerin to one patient because of anginal pain (no 16).

III Data Concerning the Patients Who Had Died

Nineteen patients, 12 men and seven women, had died at ages ranging between 28 and 86 years (Table I). The observation period was 1-37 years, being 10 years or more in 12 patients. Twelve patients died in a hospital and in eight of these cases an autopsy was performed.

On calculating the expected mortality from the year

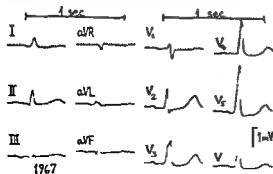


Fig 8 Case 11 Retrograde atrial activation (negative P waves in II, III and aVF) in the absence of WPW

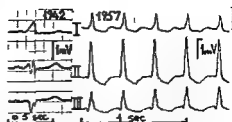


Fig 9 Case 26 1942 WPW 1957 supraventricular tachycardia



Fig 10 Case 34 WPW (1st and 3rd beats) with coupled extrasystoles (2nd and 4th beats)

of diagnoses of WPW to 1967 in each patient two patients (nos. 37 and 40) were excluded from this part of the analysis. One was born in 1863 the other in 1864 and the available tables of mortality rates do not give figures for people of such old age. In fact, they had died at the ages of 85 and 88 years (Table VI). With these exclusions the expected mortality for the remaining 45 patients was 10.0. In contrast to this the observed number of deaths was 17. This difference was significant ($P < 0.05$ χ^2) and seemed to require further analysis.

Thirty-eight patients were less than 50 years old when the diagnosis of WPW was made. The expected mortality for these patients was 4.6 whereas 11 patients had actually died ($P < 0.005$ χ^2). For patients more than 50 years of age at the time of diagnosis the calculated mortality was 5.5 and actually six patients had died ($P > 0.1$ χ^2) (Table V).

Returning to the whole material of 47 patients, 13 were 55 years or more when they died, the oldest being 86.

Table V Observed and calculated mortality in 45 patients with WPW

	Pats. < 50 y at diagnosis	Pats. ≥ 50 y at diagnosis
No. of patients	38	7
Observed deaths	11	6
Calculated deaths	4.6	5.5
Difference	6.4	0.5

Table VI Thirteen patients with WPW who had died at ages between 55 and 86 years

Case no	Sex	Age (y)	PT	HF	AH	Cause of death	Autopsy	Autopsy findings
9	♀	57	+	+	-	Cerebral thrombosis	-	
20	♂	59	-	-	-	Pulmonary embolism	+	Central pulmonary emboli thrombophlebitis of left femoral vein Slight atheromatosis of coronary arteries
21	♀	57	+	-	+	Pulmonary embolism	+	Multiple pulmonary emboli Slight myocardial fibrosis atheromatosis of coronary arteries
27	♀	86	-	+	+	Myocardial infarction	-	
30	♀	77	-	+	-	Bronchopneumonia	+	Bronchopneumonia Moderate cardiac hypertrophy moderate atherosclerosis of coronary arteries
31	♂	79	+	-	-	Cancer recti with metastases	+	Rectal cancer pleural pulmonary and supra renal metastases Bronchopneumonia Moderate cardiac hypertrophy atherosclerosis of coronary arteries
32	♀	70	+	+	-	Pulmonary embolism	+	Central pulmonary emboli left femoral vein thrombosis Slight cardiac hypertrophy Stenosis of right coronary artery
33	♂	60	+	-	+	Cancer coli postoperative state	-	
35	♂	73	+	-	-	Metastatic colon cancer postoperative state	+	Cancer coli with splenic and pancreatic metastases Acute pancreatitis Normal heart
36	♀	70	-	+	-	Myocardial infarction	-	
37	♂	83	-	+	-	Paresis cordis	-	
0	♂	80	-	uk	-	Paresis cordis	-	
	♂	55	+	+	+	Cerebrovascular insult bronchopneumonia	-	

PT paroxysmal tachycardia

HF = heart failure

AH = arterial hypertension

uk = unknown

years Table VI summarizes some of the characteristics of these patients as well as causes of death and autopsy findings. Paroxysmal tachycardia did not directly cause death in any of these cases but in two patients (nos. 9 and 33) tachycardia was present terminally.

Six patients died fairly young, the ages at death being 28, 29, 31, 34, 44 and 44 years. Of these six patients one man aged 44 committed suicide another died at the age of 35 as a result of an electric current accident. Both of these statements have been verified by forensic medical reports providing exact and unquestionable information of cause of death in both cases. Neither of these two patients had had paroxysmal tachycardia or other cardiac symptoms. The case histories of the remaining four patients who died young require further consideration and are given below.

CASE REPORTS

Case 11

Male born 1903. This patient had no history of rheumatic fever or diphtheria. He was well till 1938 when he started to have attacks of palpitation associated with precordial pain. A slight enlargement of the heart was shown by physical examination and chest roentgenogram. The ECG showed WPW. His condition remained unchanged till 1943 when he was taken to a German concentration camp where he was kept till 1945. Here he

had frequent attacks of palpitation associated with precordial pain and dyspnea. Besides he was increasingly incapacitated by dyspnea on exertion. He had long periods of diarrhea and for some time also edema of the legs. In May 1945 after his discharge from the camp he was admitted to hospital because of Bavitaminosis. A faint systolic murmur was heard over the apex but unfortunately no ECG or chest X-ray were taken. In October 1945 he had an attack of atrial fibrillation. After that he remained in fairly good shape till February 1947 when he again was distressed by exertional dyspnea and precordial pain. On admission to hospital in March physical examination showed a slightly cyanotic patient at rest, neck vein stasis and an enlarged liver but no edema. Moist rales were heard over both lungs. The arterial blood pressure was normal and no cardiac murmurs were described. The heart rate was irregular at about 100/min with a pulse deficit of 46/min. The ECG showed atrial fibrillation (Fig. 11) with occasional runs of beats with the configurational characteristics of WPW and similar to the ventricular complexes of earlier ECGs during sinus rhythm (Fig. 5). The chest X-ray showed pulmonary congestion and a considerably enlarged heart, the cardiothoracic ratio being 17/29. He was digitalized without any effect. He died suddenly on April 10, 1947. An ECG taken some time on that day showed atrial fibrillation with a ventricular rate going up to 740/min (Fig. 5).

Autopsy revealed a diffusely dilated and hypertrophied heart. Coronary arteries and valves were normal. Micro-

scopic examination showed a pronounced diffuse myocardial fibrosis, especially subendocardially and also more extensive patches of fibrous tissue. The muscle cells showed degenerative changes with vacuolization. The diagnosis of chronic myocarditis was made.

Comment

This case does not seem to be one of uncomplicated WPW syndrome. Unfortunately the exact frequency and duration of the attacks of palpitation or tachycardia are not stated in the records but long lasting attacks seem to have been rare as the patient more often complained of short lasting palpitation. During his final illness he had a poorly controlled atrial fibrillation. A slight enlargement of the heart was noted already in 1938 and this became more pronounced during the following years. It is difficult to assess the role of the B vitaminosis in this setting but it may have been of some importance. With proper therapy however signs of benign heart affection should disappear (5). Arterial hypertension was not present and coronary artery disease and valvular lesions could be ruled out on autopsy. Microscopy showed a picture which is consistent with a myocardiopathy of some kind. The etiology remains obscure. The family history is not known.

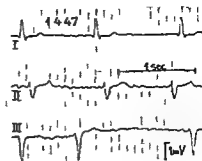


Fig 11 Case 11 Atrial fibrillation.

Case 22

Male born 1915. There was no history of rheumatic fever or diphtheria. The patient was well till 1933 when after exertion he felt dizzy and vomited. His pulse rate was 27/min. He recovered quickly and was completely well and fit till 1943 when he started to feel extremely tired and got dyspnea on exertion. He fainted 3-4 times and was taken to hospital. A faint systolic murmur was heard over the apex, the pulse rate was 50/min, and a chest X-ray showed an enlarged heart, the enlargement involving mainly the left ventricle. Most of the 12 ECGs taken showed a short P-R, broad QRS and slurring of the upstroke of the ventricular complexes (Fig. 12 (Oct. 9 1943)). Two ECGs, however, showed atrioventricular block associated with another type of QRS complexes which were

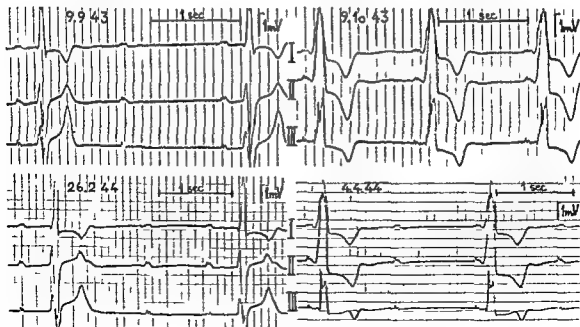


Fig 12 Case 22 Sept. 9 1943 2 1 a v block without WPW Oct. 9 1943 WPW Feb. 26 1944 3 1 a v block

with normal ventricular complexes April 4 1944 2 1 a v block and WPW complexes

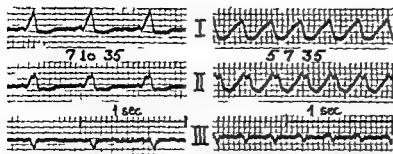


Fig 13 Case 38 Oct 7 1935 WPW July 5 1935 Regular tachycardia with abnormal intraventricular conduction

narrower On one occasion there was only a prolonged P R (0.30 sec) on another (Fig 12 (Sept 9 1943)) there was 2:1 atrioventricular conduction He was discharged without treatment

Three months later (Feb 1944) he was admitted to another hospital because of 4-5 syncopes occurring on the same day after exertion (3 football matches) His pulse rate on admission was 60/min His heart was enlarged but he was not in failure He continued to have attacks of very pronounced bradycardia and eventually died on April 10 1944 His ECGs during this admission showed 2:1 and total atrioventricular block 2:1 block was as associated with both previous types of QRS complexes (Fig 12 (Feb 26 and April 4 1944)) The autopsy showed pronounced biventricular hypertrophy whereas coronary arteries and valves were normal

The family history revealed that his sister and his father had died in a similar clinical setting at the ages of 32 and 35 years respectively and the 1 year-old daughter of his brother had fainting fits

Comment

This patient did not have attacks of tachycardia The most characteristic clinical features were those of Adams Stokes attacks due to atrioventricular block enlargement of the heart and a family history suggesting that three other members of the family might have had the same disease These features strongly suggest the diagnosis of familial cardiomegaly as described by Evans (4) Association of this disease with WPW has been reported in a few cases (8)

In this case the diagnosis of WPW was made on the basis of short P-R (0.12 sec) associated with broad QRS complexes (0.18 sec in lead II) and a slurred upstroke of QRS of 0.04-0.06 sec duration in the presence of normal sinus rhythm When atrioventricular block was present it was associated with narrower QRS complexes and disappearance of the delta waves During the final course of the disease however the WPW type QRS was also associated with 2:1 atrioventricular block but the P-R interval of the conducted beats

was invariably short (0.12 sec) as opposed to the P-R interval of 0.24-0.30 sec associated with the narrower type of QRS

Case 24

Male born 1921 This patient was completely well until 1947 when he experienced his first attack of tachycardia for which he was admitted to hospital On his arrival the tachycardia, which had lasted for 16 hours had disappeared The ECG showed typical WPW (Fig 1) Physical examination and chest X-ray were normal During the following years he had one attack every one or two years, but from 1947 he had one or two attacks a year In 1943 1945 1947 and 1951 he was readmitted for control to Medical Department B From 1947 to February 1951 his heart volume as evaluated from his chest X-ray increased from 337 ml/m to 477 ml/m² Twenty five ECGs all demonstrated WPW In 1947 he had two attacks of tachycardia in his home each lasting for 24 hours In 1948 1949 and 1950 he was admitted to a local hospital because of supraventricular tachycardia at the rate of 230/min During all these years he remained free from other symptoms and was able to work as an agricultural labourer

In August 1951 he died suddenly in his home during an attack of tachycardia which had lasted for eight hours No autopsy was performed

Comment

At the age of 31 this patient died suddenly and unexpectedly during an attack of tachycardia He had had paroxysmal supraventricular tachycardia for 9 years often associated with a high ventricular rate The maximal frequency of attacks was two per year Usually the attacks were long lasting the maximum duration being 24 hours His heart volume had been increasing somewhat but was only 477 ml/m² six months before he died All other cardiac findings were normal and he was not in failure The exact cause of death remains obscure

Case 38

Female born 1909 This patient was well till 1933 when during exertion she got an attack of tachycardia lasting

for one day. During the following two years she had 2-3 attacks of rapid heart action associated with dyspnea and precordial pain. In July 1935 she had another attack which had lasted for five days when she was admitted to hospital. On admission she was severely distressed with cyanosis and orthopnea, neck vein stasis and liver enlargement, but no edema. Moist rales were heard over both lungs. No cardiac murmurs were described. The arterial blood pressure was 95/60. The ECG showed a regular tachycardia at 230/min with broad QRS complexes (Fig. 13). The chest roentgenogram showed considerable cardiac enlargement, the cardiothoracic ratio being 16/74.5. She was running a slight fever for two weeks. She was treated with digitalis and quinidine and sinus rhythm occurred on July 14, 1935. The ECG then showed WPW (Fig. 13). In November 1935 she had a quite similar episode and was readmitted. The course and clinical findings were exactly the same.

In January 1937 she delivered a child without any cardiac complications and she remained well till October 1937 when after two weeks of tachycardia and fever she was readmitted. Physical examination disclosed neck vein stasis, liver enlargement, ascites, and edema. Pulmonary crepitation was present and the heart was enlarged. The heart rate was about 200/min. During the following weeks she deteriorated gradually. Her heart failure and tachycardia were not influenced by digitalis, quinidine and diuretics. Gallop rhythm and changing systolic murmurs were heard. The temperature was constantly elevated and a few days before death petechiae and ecchymoses developed along with bullae and furuncles. She died in December 1937. An autopsy was not performed.

Comment

This patient had extremely severe attacks of tachycardia. They were long lasting and associated with a high ventricular rate of 200-230/min and pronounced right and left heart failure. During her final illness fever, persistent tachycardia, increasing heart failure, changing murmurs and petechial skin bleedings were present and may suggest the diagnosis of bacterial endocarditis, although this was not proved by blood culture. Whether this was a secondary phenomenon in a severely ill patient with a low resistance remains unknown. The question whether this patient had a myocardial or valvular disease could not be settled either. The chest X-rays all demonstrated an enlarged heart but they were all taken during long lasting attacks of tachycardia.

DISCUSSION

The diagnostic criteria used in this paper correspond to those given by other authors (1, 6, 13). Borderline cases are found in all series. In fact Lamb states that specific interval figures for QRS

duration and PR interval cannot be given. Except in the classical form pre-excitation is best detected by comparing the abnormal record with a normal one" (7).

It has been repeatedly stressed that, as a rule P waves are normal in patients with WPW. On re-examination one patient in this series (no. 26) had negative P waves in leads II, III and aVF in the presence of normal QRS complexes. As a possible explanation of such findings which are occasionally encountered in these patients, Scherf and Cohen suggest that occasional reversed conduction to the atria during pre-excitation may induce a transient shift of the pacemaker to the a-v node (13).

The occurrence of atrioventricular block in patients with WPW is rare. The literature on this particular subject has been reviewed by Scherf and Cohen (13). It is stated that in most cases the WPW pattern disappears when atrioventricular block is present. However, in one patient (no. 22) in this series 2:1 atrioventricular block was associated with persistence of the WPW pattern in the conducted beats during his final illness. A few cases of this kind have been reported. In most of them the atrioventricular block occurred during an acute inferior wall infarction.

Paroxysmal tachycardia was experienced by 62% of the patients in this series. This is in keeping with the figures of 40-80% given in other hospital materials and in contrast to the low frequency of 13% found by Berkman and Lamb among 128 U.S. Air Force personnel (11).

Several authors state that the age at first attack of tachycardia is rarely more than 30 years (11). In this series five patients experienced their first attack at an older age (Table III).

The prognosis in the Wolff-Parkinson-White syndrome is generally thought to be good in the absence of associated heart disease and paroxysmal tachycardia (1, 11, 13, 18). On the other hand many patients with WPW and paroxysmal tachycardia are able to live normal active lives and longevity is experienced by a number of them. However, some patients are severely incapacitated by their attacks which, when long lasting and frequent, may lead to heart failure. In most series there are instances of sudden unexpected death among patients with WPW associated with tachycardia (12, 13). The reason why such patients die suddenly remains obscure. As an explanation

Scherf and Cohen suggest that an impulse conducted reversely to the atria over one a-v path way may return to the ventricles over another pathway and cause ventricular fibrillation if it arrives during the supernormal phase (13). In infants with WPW tachycardia is often life threatening as ventricular rates tend to be very high and association with congenital cardiac anomalies is fairly often seen (15).

American insurance companies consider the life table mortality rate to be increased by 25-30% for people below the age of 35 with WPW without paroxysmal tachycardia and by about 100% for people over the age of 35. In the presence of paroxysmal tachycardia the mortality rate is considered increased by 60-300% depending on the number, duration and character of the attacks (14). In Sweden neither frequent nor prolonged attacks of tachycardia cause a premium increase for a subject with WPW (12). In Denmark the premium for persons with WPW whether associated with tachycardia or not equals that for people who are five years older provided that no other signs of heart disease are present (10).

In this series the observed mortality was significantly higher than the expected mortality (Table V). This difference was almost exclusively caused by deaths occurring among the younger patients. Six deaths occurred in patients aged 44 years or less and 13 deaths in patients aged 55 years or more whereas no patients died between the ages of 44 and 55 years. In the patients who died at the age of 55 years or more all deaths were probably unrelated to WPW. Four younger patients died a cardiac death. Besides WPW one of these (no. 38) might have had and two (nos. 11 and 22) certainly had some other cardiac disease.

In a twenty-one year follow up study of 50 patients with WPW and paroxysmal tachycardia Orninus found no over mortality (12). One patient had died suddenly during an attack of tachycardia.

Berkman and Lamb have recently reported the results of a five to twenty eight year follow up study of 128 U.S. Air Force personnel (2) of whom seventeen subjects (13%) had paroxysmal tachycardia. Three deaths occurred none of which—according to the authors—was ascribed to tachycardia. One was self inflicted and another was due to an aircraft accident. It should be added that information about the third was not obtained. In the absence of paroxysmal tachy-

cardia and signs of organic heart disease pilots who had completed their training were allowed to remain on flying status.

P. D. White followed 100 cases and observed only one fatal case. Two of the 11 patients who made up the material published in 1930 by Wolff Parkinson and White have also been followed for 27 and 38 years respectively. One died at the age of 62 probably a coronary death, the other is still alive and well. Both had paroxysmal tachycardia and in both of them the frequency of attacks had decreased (3).

In conclusion it may be stated that in spite of the fairly high mortality figures in this small series of 47 patients the justification for the usual reservations made regarding the prognosis in the Wolff Parkinson White syndrome should not remain unquestioned. More systematic follow up studies preferably including other than hospital materials are needed.

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THE BEHAVIOUR OF BLOOD AND EXTRACELLULAR VOLUME IN HYPERTENSIVE PATIENTS WITH RENAL INSUFFICIENCY

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Abstract Determinations of distribution volumes of R.I.S.A. (Radio iodinated serum albumin) and bromide (as a measure of blood and extracellular volume respectively) have been performed in 35 hypertensive patients, of whom 25 had advanced renal insufficiency.

In accordance with generally accepted views the results were within normal limits in the ten patients with uncomplicated hypertension. In 10 of the 25 patients with renal failure significant elevations of either both volumes or extracellular volume alone were present.

Expansions up to 5 l of bromide distribution volume were not evident by conventional clinical examination.

There was a good correlation between blood pressure and bromide volume expansion in patients in whom the fluid excess could be removed.

It is concluded that hypertension in patients with advanced renal insufficiency may be related to fluid retention.

This fluid retention is often the result of inability of the kidney to excrete salt and water and may lead to secondary heart failure.

With progressive renal failure in chronic renal disease the handling of salt and water by the kidney is bound to become increasingly limited and disturbances of the balance in both directions may be anticipated. It is well known that in the anuric patient moderate hypertension occurs in connection with over-expansion of the extracellular fluid compartment (7-13). Observations linking water and salt retention with the production of experimental hypertension in animals are abundant. In normal human subjects aldosterone induced moderate salt retention is followed by blood pressure elevation (1). In patients with normal renal function or modest chronic insufficiency however it is generally held that no abnormalities of the body fluid compartments exist.

In order to evaluate the state of body volume

regulation in renal failure determinations were made of blood volume and bromide distribution volume in patients with advanced renal insufficiency and hypertension.

For comparison we include our results in hypertensive patients without renal functional impairment.

METHODS

Blood volume was determined with R.I.S.A. using the volumetric apparatus. The apparent distribution was corrected according to an assumed total body haematocrit value of 0.91 times the venous haematocrit (15).

The distribution volume of bromide was determined after 1 / hours of equilibration with the same apparatus according to a method described by us previously (13). The results were found to be highly reproducible in the same individual and to correlate well with expected changes in certain pathological conditions (15). We therefore take it to be a reliable approximation of the extracellular fluid volume (6). The expected "normal" values were calculated from regression curves relating our own results in a number of normal men and women to body surface (Dubois formula).

The chromogenic free creatinine in plasma was determined by means of the modified Jaffe reaction (8).

RESULTS

The main clinical findings of our patients are summarised in Table I. Patients 1 to 10 who had hypertension not complicated by renal failure serve as a comparison. Absence of cardiac failure in these patients was evidenced by the normal central venous pressure and absence of pulmonary congestion on the chest X-ray. As shown in Table II the absolute values as well as the ratio between blood and bromide volume do not differ significantly from normal.

Table V Blood and bromide volume increased

Pat no	Blood volume (l)	Deviation from normal ()	Bromide vol (l)	Deviation from normal ()	Bromide vol / blood vol
15	5.73	+11	26.5	+26	4.6
19	4.59	+22	16.6	+12	3.6
21	6.0	+25	33.4	+45	5.6
28	5.41	+14	20.2	+2.5	3.7
29	6.30	+33	23.6	+20	3.7
30	5.80	+30	18.6	+2.3	3.2
31	6.1	+16	27.4	+21	4.5
35	6.30	+26	23.6	+19	3.7
Normal	5.0	8.9	5.0	6	4.1
Normal	5.0	12	5.0	7	4.3

The second group consists of patients whose bromide volumes exceeded their calculated normal volumes by more than twice the standard deviation whereas blood volumes were normal or even lower than expected. As a consequence bromide/blood volume ratios were still (with one exception) in excess of the normal value (Table V). A striking feature was the occurrence of bromide volumes up to 5 l excess without signs of oedema.

In a third group we placed patients with increased blood volumes. Bromide volumes were

greatly increased in most of them (Table V) but in patients 28 and 30 it did not exceed the normal variation. All except one (patient 19) had elevated central venous pressures. Five of them (patients 15, 21, 30, 31 and 35) also showed clinical and roentgenological signs of left sided heart failure.

Some of the patients with increased bromide distribution volumes were subjected to dehydrating measures (salt restriction, peritoneal or extra corporeal dialysis) until their body weight was decreased by a value approximately equal to the

Table VI Blood pressure and volume measurements before and after dehydrating measures

Pat no	Blood vol (l)	Difference (l)	Bromide vol (l)	Difference (l)	Body weight (kg)	Difference (kg)	Time interval (d)	Blood pressure
18	5.99	0.69	30.5	8.1	86.5	4.3	5	160-110
	5.30		22.4		82.2			130-70
20	4.71	0.21	24.2	5.4	65.1	4	8	180-170
	4.5		18.8		61.1			140-100
20	6.0	1.5	33.4	14.2	71.2	13.5	19	180-120
	4.5		19.2		57.7			130-100
22	5.47	0.77	24.9	3	69.6	3.1	5	200-120
	4.70		21.9		66.5			145-90
23	5.75	1.32	21.4	0.8	64.5	2.8	8	190-110
	3.93		20.6		61.7			160-110
29	6.30	1.66	23.6	4.6	67.5	3	8	180-110
	4.64		19.0		64.5			140-90
31	6.1	1.0	27.4	5.9	69.5	4.8	10	170-110
	5.1		21.5		64.7			130-100
32	4.97	0.89	17.1	3.2	51.4	2.3	9	180-110
	3.86		13.9		49.1			140-80
33	5.73	1.03	26.5	7	72.2	6	20	180-90
	4.70				66.7			140-90
34	3.88	0.27	24.6	8.1	60.3	4.8	14	210-115
	4.15		16.5		55.5			130-80

* One year after the first determination

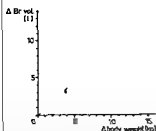


Fig 1 Relationship between change in body weight and change in bromide distribution volume in ten patients after various dehydrating measures. Dashed line represents correlation that would exist if every kg weight loss were derived from 1 l bromide distribution volume

fluid excess determined. In all instances these measures resulted in a drop in blood pressure (Table VI). The fact that the feeling of wellbeing improved and no signs of dehydration appeared confirmed that the removed amount of fluid was indeed an excess. The decrease in bromide volume correlated reasonably well with the observed weight loss (Fig 1).

DISCUSSION

Although there is general agreement in the literature that blood volume is normal in uncomplicated hypertension (11, 12, 17) there has been some discussion about the amount of extracellular fluid. Grollman (10) noted a 20% expansion of radiolabelled sulphate and mannitol distribution volumes in hypertensive patients. However de Graeff (9) and Hollander (11) could not confirm these results using radiolabelled sulphate and inulin volume measurements. During the past years the existence of a normal extracellular volume in uncomplicated hypertension has been generally accepted.

Our results are in accordance with this current opinion. Although bromide distribution volume in man is larger than those of the above mentioned substances, our findings support the usefulness of bromide volume as a clinical parameter.

Our studies indicate that in far advanced renal insufficiency with hypertension, expansion of either blood or extracellular volume or both are the rule rather than the exception. They are in accordance with earlier reports from Schriber's group (2, 3).

Boen (3) called attention to the fact that fluid

subtraction in patients on intermittent haemodialysis is often followed by lowering of elevated blood pressure. He stressed that the time interval before the blood pressure reacted to this treatment varied considerably in different patients. This may be one of the reasons why there is no general agreement as to the efficacy of fluid subtraction in these patients.

Bumberg et al (2) found elevated values for bromide and Na^+ distribution volumes in patients with far advanced renal failure both with and without intermittent haemodialysis treatment. In both groups satisfactory blood pressure control was achieved when Br^- and Na^+ distribution volumes were reduced to near normal levels. Merrill et al (14) had also shown a close correlation between sodium balance and blood pressure in bilaterally nephrectomised patients.

In the totally anuric patient treated by periodic haemodialysis any expansion of the body fluids is evidently the result of failure of excretion by the kidney. In the anephric dog blood pressure correlates with the state of hydration (16). Observations by the team of Borst (4) extensively documented that in man with intact kidneys fluid retention is followed by hypertension. This was also clearly shown in the aldosterone treated subjects of August & Nelson (1) although the authors did not comment upon this point. As all of our patients were in preterminal state (though urine volume in most of them was still maintained) the inability to excrete more than a minimal amount of salt and water resulting in positive fluid balance is the most likely explanation in this type of patient too.

It has been noted by Hollander (11) that only hypertensive patients with congestive heart failure had an expanded sulphate volume. Though heart failure may undoubtedly occur as a result of hypertension and subsequently cause fluid retention, primary fluid retention by the kidney will eventually result in congestive failure and the clinical end result may be indistinguishable. The fact that correction of the fluid excess causes the blood pressure to drop is suggestive of the fact that fluid retention presumably of renal origin was the primary occurrence.

In Hollander's study (11) six of the ten patients with malignant hypertension showed expanded sulphate volumes. In only one of them was venous pressure elevated but they all had

prolonged circulation times. These patients had little renal functional impairment. Blood volumes were not measured but were probably increased having regard to the circulation times. The authors considered them to have no impairment of sodium excretory capacity.

✓ In any case our patients shown in Table IV belonged to a clinically different group (they all had severe renal failure). They did not have increased blood volumes which in itself makes heart failure improbable. The 20 patients who showed abnormal volume determinations undoubtedly constitute a heterogeneous group and have been arbitrarily divided according to their blood volumes into the groups represented in Tables IV and V. All of these uraemic patients had anaemia, the haematocrit values ranging from 19 to 31% in patients 17-35. Only patients 15 and 16 had haematocrit values of 41 and 39% respectively. The values for total serum protein and albumin content varied among the different patients, being usually in the lower range without reaching "nephrotic" values. Patient 27 whose values for blood and bromide volume contrasted sharply (Table IV) showed the lowest albumin concentration (22 g per l) which certainly contributed to this extracellular fluid excess. In the other patients no clear correlation existed between the serum albumin level, the haematocrit value, the nature and the degree of renal insufficiency and the patterns of volume abnormalities. We believe that these and other partly unknown factors (like previous diet) although contributing to the disturbance were too manifold to permit their respective evaluation in a small number of patients.

Some of the patients in Table IV probably represent a later stage than those in Table III, whereas in those with relatively expanded blood volumes (low bromide volume/blood volume ratio) cardiac failure certainly predominated and may have contributed to the fluid retention. In favour of this assumption is that in the patients whose bromide volumes were within normal limits attempts to lower blood pressure by further fluid subtraction resulted in severe orthostatic complaints, impairment of wellbeing and some times a rise of blood urea levels. The finding of Shibagaki (18) that kidneys of patients with terminal renal failure may have elevated renin contents suggests that abnormal release of renin by

the diseased kidney may be related to hypertension even in patients with far advanced disease.

In many patients however as shown in Table VI correction of fluid excess improved the blood pressure and the general condition. It is important to realise that significant but clinically unsuspected fluid retention may be present and is often related to blood pressure elevation. In view of the grave prognosis of most patients in this group treatment of this aspect alone is not always very rewarding but from our experience it may result in remarkable subjective and objective improvement.

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HEREDITARY COPROPORPHYRIA

A Family with Unusually Few and Mild Symptoms

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Abstract A family with hereditary coproporphyrinuria from central Jutland Denmark is described. In spite of excretion of considerable amounts of coproporphyrin III with the feces but no protoporphyrin and occasionally increased urinary porphyrin as well as porphyrin precursors (PBG and/or ALA) in the urine all the affected members except one were completely free from symptoms of porphyria during the four years the family was under observation. The only member of the family exhibiting symptoms had a mild attack of acute intermittent porphyria type presumably provoked by barbiturates accompanied by pyelonephritis and colic sepsis. She constantly excreted high amounts of coproporphyrin III in her feces and periodically at least increased coproporphyrin and slightly increased uroporphyrin with the urine. In most of her siblings the excretion was intermittent and in all of them it was considerably lower than in the patient although highly elevated. Among the next generation the excretion was much lower. The observations support the concept of dominant inheritance.

In a recent paper Connon and Turkington (1) described a case of hereditary coproporphyrin (HCP) with photosensitivity and episodes of jaundice and four latent cases her father and all her three siblings. Furthermore mental disorders occurred in the family. These authors emphasize that HCP in contrast to earlier reports is far from always of a benign nature. We therefore find it appropriate to publish in some detail our studies through four years of an essentially benign form of HCP occurring in a family in central Jutland as this family has earlier been reported on only very briefly and insufficiently (3). Case history and family studies follow below. The chemical technique employed was that described in detail by With (2). The clinical examination of the family members was performed by one of us (J C L.) the chemical analyses and preparations by the other (T K. W.)

CASE REPORT

A 5 woman born Dec 8 1908 unmarried no 110 in the Danish porphyria register. Admitted on June 11 1964 to the Surgical Department of the County Hospital Holstebro for appendicitis. Since March 1964 she had suffered from sciatica like pains. On June 1 she began to take a composite mixture containing phenemal. This was followed by diffuse abdominal pains and vomiting. After transient amelioration the pains came back and she was admitted as acute patient with abdominal pain constipation and tachycardia. On June 15 it was noted that her urine was reddish and porphyrinuria was found after which she was transferred to the Medical Department on June 18. Examination then showed a blood pressure of 240/130 mm Hg = 112 and temperature 37.6 °C. The following days she gradually became mentally confused and her temperature rose to 39.4 °C on June 24. Urine examination had shown a pyelonephritis and blood culture showed growth of coli bacilli for which antibiotics were administered. Her temperature began to fall on June 25 and reached normal on June 30. Her critically high blood pressure gradually fell to between 100 and 150 systolic and between 110 and 100 diastolic. On July 6 antibiotic treatment was stopped which was followed by a slight increase in temperature until July 25 when a steep rise of temperature to 39 °C occurred. After repeated antibiotic treatment the temperature became normal within a week. Because of the high initial blood pressure she was suspected of pheochromocytoma, but studies of urinary catecholamines did not support this diagnosis. Her ECG was normal except for left axis deviation and X ray of her thorax showed nothing abnormal. Pyelography (i.v. and direct) disclosed a double renal pelvis on the left side but was otherwise normal. She was discharged on Oct 26 relieved of all her complaints except a slight uncomplicated hypertension which was not regarded as requiring specific treatment.

Porphyrin Studies on the Patient (A 3 in Table I)

After porphyrin had been demonstrated in the urine of the patient her excreta were sent to the laboratory of Svendborg County Hospital where her urine was found to contain 440 µg coproporphyrin per 100 ml and 20 µg uroporphyrin per 100 ml as well as PBG 10 and

Table I A—first generation B—second generation

A 1 died at time of investigation

A 2 died from cancer of the large intestine in 1966

A 1	♂	1905	
A 2		1906	
A 3	♀	1908	
A 4		1915	
A 5	♂	1915	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> B 51 ♂ B 52 ♀ B 53 ♂ B 54 ♂ B 61 ♂ B 62 ♀ B 63 ♀ B 64 ♂ B 65 ♀ </div> </div>
A 6	♂	1917	
A 7	♂	1920	
A 8		1923	
A 9	♂	1926	
A 10	♀	1929	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> B 101 ♀ B 102 ♂ </div> </div>

Within brackets = no chemical studies of subject in question
 Without brackets or underlining = chemical studies on urine and feces performed and found normal

— = Latent carrier i.e. abnormal values in urine and/or feces or carriers among the offspring

== = at least one attack of AIP type

ALA 0.65 mg per 100 ml, while her feces contained as much as 1800 µg coproporphyrin per g of dry substance all the porphyrin being coproporphyrin. The porphyrin from her feces was prepared as purified ester and exhibited characteristic conglomerates of very short rather coarse crystal needles and the low melting point (110°) of the crystals characteristic of coproporphyrin III ester. A family study was planned the results of which is presented in Table I.

Porphyrin Studies on the Family (Symbols see Table I)

In August 1964 the findings were as follows: A 2 normal porphyrins PBG and ALA in urine but in feces coproporphyrin 268 µg per g dry substance. A 3 patient, see above. A 4 normal conditions in both urine and feces. A 5 urine normal feces 340 µg coproporphyrin per g dry matter. A 6 urine showed negative screening test for porphyrins, PBG 0.15 ALA 0.60 mg/100 ml and feces coproporphyrin 340 µg per g dry substance. A 7 and A 8 normal findings. A 9 urine normal feces 335 µg per g dry matter. A 10 urine normal, feces 80 µg per g dry matter.

B 51–B 54 aged 22–52 years were studied in November 1964. B 51 showed normal urine but coproporphyrin 80 µg per g dry matter in feces. B 52 showed urinary PBG 0.4 and ALA 0.95 but normal fecal porphyrin. B 53 and B 54 both showed slightly elevated urinary PBG (0.3 and 0.35 mg/100 ml respectively) but otherwise normal analytical data. B 101 and B 102 aged 13 and 10 years both showed slightly elevated PBG (0.40 and 0.55 respectively) but otherwise normal findings. B 61–B 65 aged 20 to 6 years were studied in April

1965 showed normal excretion and were clinically normal.

During April 1965 A 6 was studied again and showed coproporphyrin 880 µg per g dry matter in feces, but normal urine analysis for porphyrins PBG and ALA.

During November 1965 A 7 was studied and showed PBG 0.24 and ALA 4.0 mg/100 ml in urine the feces were not studied on this occasion.

During October 1966 A 3 showed PBG 0.63 mg/100 ml but normal ALA and coproporphyrin in feces 1765 µg per g dry matter. A 4 showed PBG 0.75 mg/100 ml on this occasion but normal ALA and fecal porphyrins. A 7 showed no findings in urine but coproporphyrin in feces 155 µg per g dry matter. A 8 exhibited normal urine and feces values. A 10 showed normal ALA but slightly elevated PBG (0.30 mg/100 ml) and fecal coproporphyrin 430 µg per g dry matter.

During May 1967 A 10 was studied again and showed normal urinary PBG negative screening test for porphyrins in urine and ALA 0.75 mg/100 ml. Fecal coproporphyrin was 650 µg per g dry matter.

During July 1968 A 3 showed slightly elevated PBG (0.36 mg/100 ml) and normal ALA urinary coproporphyrin 70 and uroporphyrin 15 µg per 100 ml. Her fecal coproporphyrin was 1400 µg per g dry matter. A 6 had normal urine but fecal coproporphyrin 245 µg per g dry matter. A 10 showed PBG 0.95 but normal ALA and negative screening test for urine and fecal porphyrins.

During the summer of 1964 one of us (J. C. L.) contacted the family members A 7, A 4, A 5, A 6 and A 10 as well as eleven of the members of the second generation B 51–B 54, B 61–B 65, B 101 and B 102. None had any complaints suggesting acute porphyria attacks and no photosensitivity, skin fragility or abnormal pigmentations.

DISCUSSION

The essential benignity of the type of HCP prevailing in this family is evident. It is noteworthy that patient A 3 had highly elevated coproporphyrin in feces on all occasions when it was determined and that also her urinary coproporphyrin and to a lesser degree uroporphyrin was elevated on the two occasions when it was measured. Her PBG was also increased on all three occasions when it was measured but ALA was only increased on the first study during her attack. Some of her siblings showed increased ALA with normal PBG (A 6 in 1964, A 10 in 1967) while others had normal ALA with increased PBG (A 4 in 1966, A 10 in 1966 and 1968 as well as A 3 herself in 1966 and 1968). In A 4 the fecal porphyrin screening test was negative on the two occasions when she was studied (1964 and 1966) for which reason the character of this disease cannot be reliably determined on the basis of fecal analysis alone. It came on one occasion

(1966) she had definitely elevated urinary PBG. In some of the latent cases (*A 6*) the fecal porphyrin was constantly elevated as found in patient *A 3* while in others (*A 10*) the fecal porphyrin excretion was intermittent.

The excretion in members of the second generation—aged 22–6 years—was much lower than in the first generation and only in one of them was the fecal coproporphyrin elevated and only to a very moderate degree contrary to the urinary excretion of precursors which was slightly elevated in a high percentage of the few members of the second generation studied: an elevation just above normal limits.

It is surprising that only one (*A 8*) of the members of the first generation subjected to study showed normal conditions in all the tests employed (screening for porphyrins in urine and feces, ion exchange chromatographic determination of PBG and ALA, quantitative porphyrin analyses if the screening tests were positive). In the second generation six of the 11 subjects studied exhibited minor increases either of the excretion of porphyrin precursors with urine or of fecal porphyrins. The same high incidence of latent cases was found by Connon and Turkington (1)—all three siblings and the father.

The only porphyrin present in the feces was coproporphyrin; protoporphyrin was never found in analyses including thinlayer chromatographic studies (4–6). The porphyrin was prepared as crystalline ester from the feces of *A 3*, *A 5* and *A 10* and in all three the crystals were ball like conglomerates of short needles (hedgehogs) with melting point between 120 and 140°C typical of coproporphyrin III tetramethyl ester.

This family study differs from most others in being continued over several years and has disclosed intermittent excretion in several of the latent carriers. Although fecal porphyrin excretion is the dominant chemical symptom in this family it is not constant and sometimes the feces were normal while the urinary excretion of precursors was elevated. In one (*A 4*) her status as a carrier could only be found on urinary analysis as her only abnormality was a definitely increased PBG on one occasion.

The findings stress the necessity of always studying both urine and feces and of employing ion exchange analysis for the precursors as previously made clear by one of us (3–5).

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SERUM IMMUNOGLOBULINS AND ORGAN SPECIFIC CELLULAR HYPERSENSITIVITY IN ULCERATIVE COLITIS AND CROHN'S DISEASE

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Abstract The serum concentrations of IgG, IgA and IgM have been measured in 27 patients with ulcerative colitis and in 23 patients with Crohn's disease. The concentration of IgG was significantly higher in ulcerative colitis than in Crohn's disease but the examination did not show any major differences between the two conditions or alterations from the normal. In Crohn's disease the serum concentration of IgG was lower in patients treated than in patients not treated with glucocorticoid. A similar difference was not found in ulcerative colitis.

In a parallel study on the same patients the *in vitro* inhibition of leucocyte migration induced by jejunoileal and colonic mucosa components was examined. A reactivity which is probably an *in vitro* correlate to organ specific cellular hypersensitivity to intestinal mucosa components, was demonstrated in a majority of the patients with ulcerative colitis, whereas patients with Crohn's disease did not differ significantly from controls. There was no correlation between the serum concentration of the various immunoglobulins and the result of the leucocyte migration test.

As important fluctuations in production, catabolism, extravascular deposit, and loss of immunoglobulins may be concealed behind comparatively normal serum immunoglobulin concentrations, more conclusive results may be gained from examinations which include metabolic studies of the immunoglobulins.

The etiology of ulcerative colitis and Crohn's disease is still obscure but immunological aspects have become increasingly important in pathogenetic considerations (7, 8). Often a distinction between the two conditions can be drawn on the basis of conventional nosographic criteria, but if only the colon is involved the classification is difficult and may be impossible.

If however a classification is made according to existing criteria ulcerative colitis seems to be associated with a hyperactive immune organ (4,

5, 12) whereas Crohn's disease is possibly associated with reduced immunological responsiveness (1, 9, 12). Although there is no general agreement on this hypothesis (6) it was found of interest to examine whether a difference of immunological reactivity is reflected in the absolute or relative serum concentrations of the major immunoglobulins IgG, IgA and IgM.

Further, as organ specific cellular hypersensitivity to intestinal mucosa components is generally not found in Crohn's disease but appears to be an immunopathological feature of ulcerative colitis (1) it was resolved to examine the occurrence of cell mediated auto-reactivity and its correlation to the immunoglobulin pattern by means of leucocyte migration tests in the same patients.

MATERIAL

The differential diagnosis between ulcerative colitis and Crohn's disease was based upon clinical features, radiological examination, recto-sigmoidoscopy and histopathological examination of biopsy specimens or tissue removed by operation. Cases which could not be classified with certainty were excluded.

Ulcerative colitis was diagnosed in 22 patients: ten females (age 10-66 average 36 years) and 12 males (age 13-63 average 35 years). All cases showed signs of activity with haemorrhagic diarrhoea, fever, abdominal pain, hypersedimentation, and leucocytosis. In four patients subtotal colectomy had previously been undertaken. Nine patients received treatment with glucocorticoids at the time of study.

Crohn's disease was diagnosed in 23 patients: 13 females (age 16-65 average 37 years) and ten males (age 15-74 average 35 years). All cases were more or less active with diarrhoea, abdominal pain, fever and



Fig 1 The serum immunoglobulin in 22 patients with ulcerative colitis compared with normal controls. The absolute values are given in the text.

hypersensitization. In six cases the changes were located in the terminal ileum in 11 cases only the large bowel was involved and six had a combined ileo-colic involvement. In five patients intestinal resection with ileo transversostomy had been performed. Six patients received treatment with glucocorticoids at the time of study.

METHODS

The concentrations of IgG, IgA and IgM in sera stored at -18°C for 2 months–2 years were estimated by electrophoresis in antibody containing agarose (13, 14). Human Standard Serum Op no 166 (Behringwerke Marburg, Lahn, Germany) was used as quantitative reference. The rabbit antibodies used were antihuman IgG (Dakopatts Ltd, Copenhagen), antihuman IgA and antihuman IgM (Behringwerke Marburg, Lahn, Germany). The serum levels of IgG, IgA and IgM were determined in 44 healthy members of the medical staff (average age 33 years) and 55 blood donors (average age 39 years). The distribution proved to be positively skewed but the log transformed values showed a normal distribution. In all the calculations therefore log values were used. The 95% range was for IgG 7.2–15.1 g/l (mean 10.4 g/l); for IgA 0.74–3.06 g/l (mean 1.50 g/l) and for IgM 0.23–1.33 g/l (mean 0.56 g/l).

Leucocyte migration test (LMT). Organ-specific cellular hypersensitivity was estimated by means of the leucocyte migration test (LMT) which has been described in detail elsewhere (3, 11). Washed peripheral leucocytes are allowed to migrate for 24 hours from the open end of a 1.4 mm capillary tube along the plain bottom of a 1 ml tissue culture chamber. The circular migration area is measured by planimetry. The migration areas in a series of cultures without antigen are put in relation to the migration areas of a parallel series of antigen-containing cultures. The migration index (MI) hereby calculated indicates an inhibition if the value is below and a stimulation if the value is above unity.

Pooled extracts of fetal colonic or jejunoileal mucosa were used as antigens. Extracts of fetal liver and kidney were used as controls to ensure organ-specificity. The tissue extracts were standardized by protein determination. The concentration of intestinal mucosa antigen in the cultures was as routine 40 μg protein per ml culture medium.

The MI values of 55 controls examined with intestinal

mucosa extracts showed a normal distribution. The 95% range with colon mucosa (mean 0.95 ± 2 s.d.) was 0.79–1.11 with small intestinal mucosa (mean 0.94 ± 2 s.d.) 0.78–1.10.

RESULTS

Serum levels of IgG, IgA and IgM

The serum levels of IgG, IgA and IgM are presented in Figs 1 and 2.

In ulcerative colitis the mean concentration of IgG was 12.4 g/l (95% range 5.5–27.8 g/l) of IgA 1.33 g/l (95% range 0.38–4.63 g/l) and of IgM 0.54 g/l (95% range 0.18–1.60 g/l). The mean serum concentration of IgG is significantly different from the controls ($p < 0.05$). The standard deviations of IgG ($p < 0.01$) and IgA ($p < 0.05$) are different from the normal and there is a positive correlation between the serum concentrations of IgG and IgA ($p < 0.01$).

In Crohn's disease the mean concentration of IgG was 9.7 g/l (95% range 4.4–21.5 g/l) of IgA 1.25 g/l (95% range 0.36–4.33 g/l) and of IgM 0.49 g/l (95% range 0.17–1.46 g/l). The mean values do not differ significantly from the controls ($p > 0.05$). According to variation analysis the deviations in the IgG ($p < 0.01$) and IgA concentrations ($p < 0.01$) are significantly different from the normal. As in ulcerative colitis there is a positive correlation between the serum concentrations of IgG and IgA ($p < 0.05$).

A comparison between the serum concentration of IgG, IgA and IgM in ulcerative colitis and Crohn's disease shows a significantly higher serum concentration of IgG in ulcerative colitis ($p < 0.05$) but no difference as far as IgA and IgM are concerned.

Leucocyte migration test (LMT)

The migration indices in the two groups of patients appear from Fig 3.

In accordance with previous observations (1)

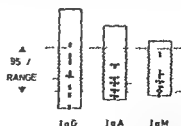


Fig 2 The serum immunoglobulins in 33 patients with Crohn's disease compared with normal controls. The absolute values are given in the text.

it was found that specific inhibition of the migration was induced by jejunoileal as well as colonic extracts. The lowest value observed in each patient is indicated in the figure.

In ulcerative colitis the mean MI (0.70) was significantly different from the normal ($p < 0.001$) and from the Crohn group ($p < 0.001$). Six patients had migration indices within the normal range.

Comparison between serum immunoglobulin levels and the leucocyte migration inhibition

In Fig. 4 the migration indices and the serum immunoglobulin levels in ulcerative colitis are compared. No significant correlation exists between the MI and the serum concentration of IgG, IgA and IgM ($p > 0.05$). The group of patients under glucocorticoid treatment does not differ from the rest as regards immunoglobulin levels and migration indices ($p > 0.05$).

In Fig. 5 the migration indices and the serum immunoglobulin levels in Crohn's disease are compared. No significant correlation exists between the MI and the serum concentration of IgG, IgA and IgM ($p > 0.05$). Patients treated with glucocorticoid had significantly lower serum concentration of IgG ($p < 0.01$) while IgA and IgM as well as migration indices did not differ from the remainder. The patients with affection exclusively of the terminal ileum did not differ significantly from the patients with colonic or combined ileo-colic involvement as regards serum concentration of the three immunoglobulins and migration indices.

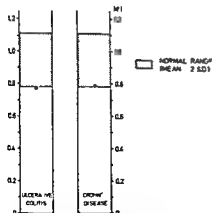


Fig. 3 The leucocyte migration indices in 22 patients with ulcerative colitis and 13 patients with Crohn's disease compared with normal controls.

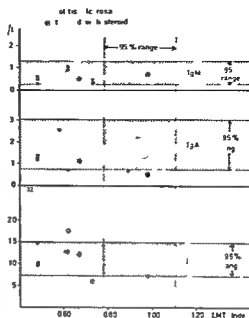


Fig. 4 The relation between leucocyte migration indices (x) and the serum concentrations of IgG, IgA and IgM (y) in 22 patients with ulcerative colitis. The patients treated with glucocorticoid are indicated in the figure.

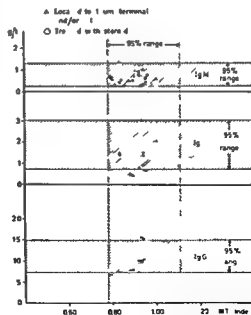


Fig. 5 The relation between leucocyte migration indices (x) and the serum concentrations of IgG, IgA and IgM (y) in 23 patients with Crohn's disease. The location of the disease as well as treatment with glucocorticoids are indicated in the figure.

DISCUSSION

Previous studies of the serum protein pattern in patients with ulcerative colitis and Crohn's disease have shown that the gamma globulin level estimated by paper electrophoresis may be above within or below the normal range (4-5-10) and a similar variation of IgG levels should be expected (14). The IgG as well as the IgA values showed a greater deviation from the normal mean than the controls and the study further showed a higher level of serum IgG in ulcerative colitis than in Crohn's disease.

The serum concentration of an immunoglobulin is an effect of a dynamic balance between on the one hand production and on the other destruction/loss or extravascular deposit of the protein. In ulcerative colitis or Crohn's disease this balance is not influenced in a way which is reflected in typical consistent changes of the serum concentrations of IgG, IgA and IgM. This finding however does not exclude the possibility that profound changes in the immunoglobulin turnover may exist.

The importance of parallel metabolic studies is clearly seen from examinations of IgG turnover in patients with Crohn's disease (2) which showed that a considerably increased production and degradation was concealed behind normal or slightly abnormal serum levels of IgG. It cannot be excluded that similar marked changes of IgG turnover exist in ulcerative colitis and in both conditions IgA and IgM may be involved as well. This however can only be decided on the basis of metabolic studies with pure preparations of these compounds. Such studies are presently being undertaken and might serve to elucidate further the immunopathological difference between ulcerative colitis and Crohn's disease.

The results of the LMT using extracts of fetal colonic and small intestinal mucosa confirm the existence in ulcerative colitis of an organ-specific cellular reactivity which is probably *in vitro* correlate to cellular hypersensitivity to intestinal mucosa components although the reaction is not present in all cases. A similar reactivity is a rare feature of Crohn's disease also in cases with colonic involvement and seems to be another example of different immunological reactivity in the two conditions. The lack of correlation between the capacity for organ-specific cellular reactivity and the serum concentrations of the

various immunoglobulins was not unexpected but does not allow any final conclusion until further studies of the metabolic pattern of the various immunoglobulins have been undertaken.

Glucocorticoid treatment did not seem to influence the serum immunoglobulins in ulcerative colitis whereas in Crohn's disease the serum concentration of IgG during glucocorticoid treatment was significantly lower than in untreated patients. This finding might indicate that glucocorticoids influence the IgG metabolism differently in ulcerative colitis and Crohn's disease. Organ-specific cellular hypersensitivity as measured by the LMT was not significantly influenced by glucocorticoid treatment.

The conclusion of the present study is that although marked alterations of the production/catabolism/loss or deposit of immunoglobulins should be expected in ulcerative colitis and Crohn's disease such disturbances if present do not induce any characteristic change of the absolute and relative concentrations of IgG, IgA and IgM in the serum and that further information has to be gained from examinations which include metabolic studies of the individual immunoglobulins.

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DISSEMINATED INFECTION CAUSED BY MYCOBACTERIUM KANSASII

Report of a Case and Brief Review of the Literature

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Abstract A case of disseminated visceral infection with *Mycobacterium kansasii* in a previously healthy 23 year old woman is reported. The clinical picture was dominated by fever granulocytopenia and weight loss. Later severe pancytopenia and splenomegaly were outstanding. The course was fatal in spite of therapy with antituberculous drugs. The association of blood dyscrasias in disseminated mycobacterial infections in man is pointed out. The literature is briefly reviewed.

The so called atypical mycobacteria which were originally considered saprophytes or commensals nonvirulent for man have proved to be the cause of both pulmonary and extrapulmonary infection. Pulmonary lesions are by far the most frequent but localized extrapulmonary infection such as suppurative cervical adenitis in children (5 29) cutaneous infection (6 17) and bone and joint involvement (5 6 15) have been reported. Disseminated infection due to these organisms has been reported (3 8 14 16 18 20 23 24 27 28 30 31 32). In cases of disseminated disease morphological changes in peripheral blood and hepatosplenomegaly are frequent findings and it is often presumed that the patients have a primary disease in blood forming tissues. The purpose of this paper is to report on a case of disseminated infection with *Mycobacterium kansasii* (Runyon Group I photochromogenic) (22) associated with pancytopenia and hepatosplenomegaly and to review briefly the literature. To our knowledge this is the first case to be reported from the Scandinavian countries.

CASE REPORT

A 23 year-old married woman was in April 1967 transferred from the Hospital of Infectious Diseases to the Department of Medicine Sahlgrenska Hospital Göteborg because of persistent fever anemia and granulocytopenia.

Since adolescence she had inclined to have a mild anemia probably due to large menstrual blood losses. Parturition 5 and 2 years before was normal. In the spring of 1966 she had a spontaneous abortion. Otherwise the past history was negative. Since childhood she had been living in Göteborg and she had never been abroad. There was no familial occurrence of tuberculosis nor was the disease known to exist in her immediate environment.

At the beginning of March 1967 there was an onset of cough without expectoration, fatigue anorexia, mild jaundice and fever to 38.5 °C. On March 30 she was admitted to the Hospital of Infectious Diseases where physical examination revealed a moderately icteric female who did not appear acutely ill. The lungs were clear. There was no lymphadenopathy the liver and spleen were not palpable but X ray examination showed a slight increase in the size of the spleen. Chest X ray showed bilaterally small infiltrations in the hilar regions. The laboratory findings were hemoglobin 10.0 g/100 ml, hematocrit 30%, RBC 2.9 mill./mm, reticulocytes 2.4%, WBC 1400/mm and a platelet count of 190 000/mm³. A bone marrow aspiration did not yield enough material for evaluation. ESR was 87 mm/h. Total bilirubin 4.5 mg/100 ml alkaline phosphatase 111 Buch Buch units (normal 2-8) thymol turbidity test, SGOT and SGPT were normal. Serum haptoglobin 247 mg/100 ml serum iron 55 µg/100 ml and TIBC 375 µg/100 ml. Direct and indirect Coombs tests were negative. There were no cold agglutinins in the serum. Complement fixation tests for enterovirus and PPLO were negative. Before institution of therapy eight blood cultures were performed and all proved sterile. In spite of treatment with antibiotics (penicillin, tetracycline, streptomycin) fever (38-39 °C) anemia and granulocytopenia persisted but jaundice disappeared and chest X ray returned to normal.

Table I Results of test for *Toxoplasma gondii* antibody

Date of collection	Sabin-Feldman dye test	Complement fixation test
March	31	1/500
April	10	1/500
May	2	1/250
May	3	1/250
May	23	1/32
July	19	1/250
		neg

On April 21 she was transferred to the Department of Internal Medicine. At examination she appeared chronically ill. The liver edge was palpated 2 finger breadths beneath the right costal margin on deep inspiration. Other wise the physical findings were negative. Laboratory tests revealed hemoglobin 10.1 g/100 ml hematocrit 35% RBC 4.0 mill/mm³ reticulocyte count 4.0% WBC 1900/mm³ with a percentage of 6 stabs, 68 segmented neutrophils, 1 eosinophile, 1 monocyte and 24 lymphocytes. The platelet count was 156 000/mm³. The urine sediment and serum creatinine were normal. The serum haptoglobin was 10 mg/100 ml and the ESR 50 mm/h. Repeated tests for occult blood in the stools were negative. Serum iron 60 µg/100 ml and TIBC 330 µg/100 ml. The serum vitamin B₁₂, whole blood folic acid and serum folic acid levels were normal. Total bilirubin and thymol turbidity tests were normal but alkaline phosphatase was substan-

tially elevated (39 Buch-Buch units). SGOT and SGPT were mildly elevated. Bromsulphthalein retention after 45 min was 16%. The total serum protein was 5.7 g/100 ml with albumin 2.3 g/100 ml α_1 -0.5 α_2 -0.9 and γ -globulin 1.6 g/100 ml. Serum immunoelectrophoresis was normal. Direct and indirect Coombs tests were negative. Repeated LE cell tests were negative. Serological tests for syphilis, typhoid and brucellosis were negative. No cold agglutinins were found. Complement fixation tests for adenovirus, ornithosis and Coxsackie B viruses, and for *Histoplasma capsulatum* were negative. Paul Bunnell's test was negative. Dye tests and complement fixation tests for *Toxoplasma* antibodies were positive (Table I). Endometrial tissue, liver tissue and blood were inoculated intraperitoneally into 6-week-old mice but *Toxoplasma gondii* was not isolated. The Mantoux reaction (1:1000) was positive and the histoplasmin skin test negative. Repeated blood cultures were sterile.

Sternal marrow aspirations performed on April 14, June 12 and July 13 yielded sparse material and the smears showed hypocellularity probably due to the admixture of peripheral blood. There was a relative increase in erythropoiesis but a marked decrease in granulopoiesis. The average differential count of the bone marrow smears showed a percentage of 3 proerythroblasts, 53 erythroblasts, 1 myeloblast, 8 myelocytes, 19 metamyelocytes and granulocytes, 13 lymphocytes, 1 monocyte and 2 plasma cells. Histological examination of biopsy specimens from spinal processes however revealed a hypercellular marrow practically without fat. There was a marked erythroid hyperplasia, normal number of megakaryocytes but a granulocytic hypoplasia. There was a discreet increase in the amount of reticulum fibrils. Granulomatous lesions were not found.

She remained in the hospital until she died on September 29. The course of her illness is illustrated in Fig 1. Initially the clinical picture was dominated by fever (38.0–39.0°C), malaise, nonproductive cough, progressive anemia and granulocytopenia. The primary suggestion was that she had a chronic infectious disease or a systemic granulomatous disorder such as Hodgkin's disease or both. Laboratory investigation for tuberculosis was started on May 5 (Table II) and prednisolone 40 mg orally per day was instituted on May 11. On May 27 a morbilliform nonpruritic skin eruption appeared over the whole body, persisted for two weeks and then disappeared. Biopsy of the skin lesions was not performed. Examination of a liver biopsy specimen taken on May 19 revealed discreet infiltration of periportal spaces with lymphoid cells and mild fatty metamorphosis. A few small granulomata of epithelioid histiocytes, some lymphocytes and polymorphonuclear leukocytes without central necrosis were found. Sections stained for acid fast bacilli (Ziehl-Neelsen) were negative.

At the end of May she again developed jaundice with a bilirubin level of 3.8 mg/100 ml. At the same time there was a fall in the hemoglobin concentration, a rise in the reticulocyte count, a low haptoglobin level and spiking fever. The anemia required blood transfusion. Because of the suspicion of disseminated tuberculosis, streptomycin, isoniazid and p-aminosalicylic acid were instituted on the 1st of June. During this therapy there

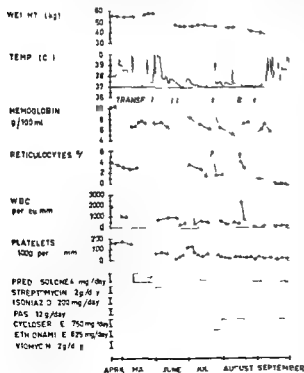


Fig 1 Course of the disease and treatment

as a transient improvement in her condition. The fever abated and she felt better. The asterus disappeared. Later, however, she continued to deteriorate. Liver enlargement remained constant but the spleen increased and was finally palpable three finger breadths below the costal arch. There was no lymphadenopathy. Numerous chest X-ray examinations were negative. X-ray examination of the gas routine spinal tract and skeleton in ravenous pyelography and cholangiography were normal. Thrombocytopenia was persistent. In June she developed a marked thrombocytopenia. After a transient rise in the platelet count she again became thrombocytopenic during July and the following months. The serum alkaline phosphatases were permanently markedly increased and the SGOT and SGPT levels slightly elevated. There was a successive fall in the serum albumin concentration (2.0-1.5 g/100 ml) and a moderate increase in the gammaglobulin level. Specimens of gastric lavage, stool, and fine needle aspiration from the spleen taken in May showed growth of uncharacteristic acid fast rods on Löwenstein-Jensen medium at the 4 week reading. The guinea pig inoculations were all negative (Table II). The organism was later identified as a photochromogenic mycobacterium (Runyon group I *Mycobacterium kansasii*). Antibiotics showed resistance to streptomycin, isoniazid and para-aminosalicylic acid. These drugs were therefore discontinued and the patient was started on cycloserine and ethionamide on July 24. The reaction to therapy was unremarkable. On August 14 minute pleural effusions were found bilaterally and cultivation of pleural fluid showed growth of *Mycobacterium kansasii*. At the beginning of September small parenchymal infiltrations were seen in the left hilar region but disappeared on X-ray control one week later. She had a pronounced pancytopenia and required blood transfusions. The peripheral differential count showed a few metamyelocytes and a few nucleated red cells but was otherwise unremarkable. Her emaciation was progressive and she refused further therapy with cycloserine and ethionamide. Two weeks after discontinuing therapy she developed high spiking fever and the therapy was re-instituted. Her condition, however, became worse; she became confused, suffered from respiratory distress and died on September 29.

Autopsy findings

At autopsy gross examination revealed marked emaciation. There was 100 ml clear fluid in the right and 300 ml slightly hemorrhagic fluid in the left pleural cavity. Small granular fibrin deposits were seen on the diaphragmatic surfaces of the visceral and parietal pleura on the side. The lungs were somewhat small and firm with ematous cut surfaces. No granulomata, infiltrates or abscesses were found in the abdominal cavity; there was 50 ml slightly hemorrhagic ascites but no sign of peritonitis. The liver was moderately enlarged (160 g) but surface was smooth and light brown in color. No infiltrates were seen. The spleen was greatly enlarged (350 g) soft and of a dark red-brown color. The capsule was thickened and there were fibrous adhesions to the omentum. On the cut surfaces of the spleen many pea-sized grayish soft foci were seen. Numerous soft, enlarged nodes up to the size of a marble were found para-

Table II Bacterial findings from clinical specimens

Date	Specimen	Acid fast organisms on direct smears, copy	Growth of acid fast organisms on L-J medium	Guinea pig inoculation
May 5	Urine	-	-	-
May 13	Urine	N	C	-
May 16	Urine	N	-	-
May 17	Urine	N	-	-
May 19	Gastric lavage	-	+	-
May 20	Endometrial tissue	-	-	-
May 23	Gastric lavage	N	C	-
May 24	Stool	N	C	-
May 24	Urine	N	C	-
May 26	Urine	N	-	-
May 27	Gastric lavage	-	-	-
May 27	Stool	N	-	-
May 27	Urine	N	-	-
May 27	Fine needle aspirate from spleen	-	+	-
May 31	Stool	N	C	-
May 31	Urine	N	C	-
June 1	Urine	N	C	-
June 2	Urine	N	-	-
June 14	Stool	N	C	-
July 14	Gastric lavage	N	+	-
July 14	Stool	+	C	-
July 14	Urine	-	C	-
July 15	Gastric lavage	+	+	-
July 15	Urine	-	C	-
July 17	Gastric lavage	N	C	-
July 17	Stool	N	C	-
July 18	Stool	-	C	-
July 22	Stool	-	C	-
Aug 18	Pleural fluid	-	+	-

N = not examined C = contaminated acid fast organisms not found

aortally in the abdomen, mainly at the level of the superior mesenteric and renal arteries. In some of them necrotic soft centers were seen. An enlarged lymph node with a necrotic center was found in the portal area adjacent to the main bile duct. Numerous enlarged lymph nodes up to 1 cm in diameter but without gross necroses were also found in the mediastinum para-aortally and paratracheally and along the carotid vessels. The bone marrow of the sternum ribs and the spine was grossly normal. Red bone marrow was also found in the femur shafts. The remaining organs, including the brain, the genito-urinary and gastrointestinal tracts, were unremarkable.

On microscopic examination the lungs showed vascular congestion with pulmonary edema. In the left lung small non-specific bronchopneumonic infiltrates were seen and were most numerous in the lower lobe where the pleura on the diaphragmatic surface was covered with fibrin deposits. No granulomas or acid fast bacilli were found in the lungs. The liver showed vascular congestion and a marked fatty infiltration which was most pronounced in

the periphery of the lobuli. Some scattered tiny necroses surrounded by a few plasma cells, lymphocytes and macrophages were found. No epithelioid cell infiltrates resembling those previously seen in the liver biopsy specimen and no acid fast bacilli were found. In the spleen several large necroses were seen. Most of them were granular containing necrotic cells and cell debris and did not show granulomatous reaction. Some of the necroses however were of a caseous structureless type and well demarcated with a fibrous periphery containing epithelioid cells and a few giant cells. The latter contained few nuclei and were of a non specific foreign body type. Langhans cells were not seen. A moderate amount of acid fast bacilli in and around the necroses was found. They were large characteristically beaded often lying in chains intra as well as extracellularly. In the lymph nodes from porta hepatis, para aortally in the abdomen and mediastinum and bilaterally from the neck necroses of variable degree were found. The necroses were granular with very little granulomatous reaction and without fibrosis. Occasional infiltrates of epithelioid cells which were most pronounced in the portal node were found. No Langhans cells but some giant cells with the appearance of large histiocytes with a few nuclei were seen. In all of the examined lymph nodes many acid fast bacilli were found most numerous in the portal and the abdominal para aortal lymph nodes.

The bone marrow from sternum, ribs, lumbar spine and even femur shafts was highly cellular. The picture was dominated by the myelopoiesis with predominantly immature myeloid cells. Megakaryocytes appeared in ordinary amounts. Some large somewhat atypical histiocytes were seen but no evidence was found of hematological malignancy. No granulomata and no acid fast bacilli were found.

In brain, kidneys, small bowel, pancreas, myocardium, adrenal, thyroid and hypophysis, nothing pathological was found on histological examination.

Culture of spleen, liver, abdominal and thoracic lymph nodes, ascitic fluid, lungs, pleural fluid, brain and cerebrospinal liquor yielded growth of photochromogenic, Runyon group I mycobacteria.

Bacteriological and serological findings

The primary isolates as well as subcultures from the different clinical and autopsy specimens all had a colony morphology on Löwenstein-Jensen medium characterized by a smooth sometimes confluent growth after 2-6 weeks incubation at 37°C. The color was pale beige when the incubation was performed in the dark. Subcultures did not show any visible growth after 7 days incubation at room temperature. Acid fast staining with Nachitblau according to Haffberg's method revealed on microscopic examination acid fast, somewhat large and often beaded or banded rods with little or no tendency to cord formation.

The macroin test according to Pekruze and Asmus-Garschagen (11) was negative and the strain was resistant to furan-carboxylic acid hydrazide at a concentration of 25 µg/ml in Löwenstein-Jensen medium. (11) Virulence test on guinea pigs performed with 1 mg moist weight bacteria inoculated subcutaneously in the inguinal region was negative at 6-week sacrifice.

Sensitivity tests revealed that the strain was resistant to p-aminosalicylic acid, streptomycin, isoniazid and kana-

mycin, moderately resistant to thiosemicarbazone but sensitive to cycloserine, viomycin and ethionamide (resistance ratio values >8.8 ~8.8 4 1 1 and 1/16 respectively as compared with H337Rv). Exposure to the light of a 40-watt lamp at a distance of 50 cm for 60 min turned the colonies bright yellow after 1-2 hours subsequent incubation in the dark. Nitrate reduction test was positive, arylsulphatase test negative and the amidase activity pattern was that of a typical Runyon group I bacterium (12).

With respect to the aforementioned findings the strain was classified as a mycobacterium belonging to the photochromogenic Runyon group I species *Mycobacterium kansasii*. (Dr Ingemar Juhlin at the Department of Bacteriology, Malmö General Hospital confirmed this result).

To determine the possible presence of circulating antibodies against mycobacteria a serum sample had been taken in July and was tested according to Ouchterlony immunodiffusion in gel technique (ID) by means of 1° mycobacterial antisera (concentrated culture filtrates). These antisera represented the following six species: *M. bovis* var BCG, *M. kansasii*, *M. scrofulaceum*, *M. avium*, *M. balnei* and *M. fortuitum*. The test serum gave positive precipitation reactions with all antisera except those prepared from the species *M. fortuitum*. Comparative ID analyses revealed a total of four different precipitins three of which were common to two or more of the mycobacterial species used for the tests. The fourth precipitin was detected only at testing with the *M. kansasii* antigen. To determine whether this precipitin could have been produced against a *M. kansasii* specific antigenic factor a comparative analysis with a *M. kansasii* reference precipitation system was performed. The reference system used had earlier been well characterized in regard to the immunospecificity of the precipitates forming its pattern. The comparative test revealed that the precipitate formed by this fourth precipitin and the *M. kansasii* antigen coincided with a precipitate in the reference spectrum labelled 4. The pertinent antigenic factor 4 has so far been demonstrated only in *M. kansasii* strains according to ID analyses of about 250 strains representing the majority of mycobacterial species. The finding of the precipitin 4 suggests that the patient was or had been infected with *M. kansasii*. One of the aforementioned three precipitin cross-reacting with several mycobacterial species was characterized anti-delta (19). This precipitin has been found in sera from healthy individuals as well as in patients infected with mycobacteria. Immunoglobulin classification of anti-delta from different origins had shown that this precipitin in sera from healthy individuals belongs to the IgM type while that from infected persons may be either of the IgM or IgG type depending on the amount of antigen stimulus and/or the duration of disease. The anti-delta in the present case was of the IgG type suggesting that this antibody was actually caused by a mycobacterial infection.

Skin test with *M. kansasii* sensu was never carried out on the patient but her family was tested in March 1968 at a routine dispensary control with the following result: PPD kansasii 5 TU husband, age 74, 8 x 8 mm, son, age 6, negative, son, age 3, 4 x 4 mm. Chest X-ray was negative and tuberculin test positive for all three.

COMMENT

Over the past ten years atypical mycobacteria (e.g. mycobacteria other than *M. tuberculosis*, *M. bovis* and *M. leprae*) have been increasingly recognized as causing infection in man. Runyon has divided these organisms into four groups on the basis of pigment formation and rate of growth (22). There is a wide regional variation in the species causing human disease. The most common site of infection is the lungs. Recent reports show that an appreciable number of patients admitted to tuberculosis services have pulmonary lesions due to atypical mycobacteria. They usually respond to therapy and have a benign course.

Disseminated disease with visceral involvement due to atypical mycobacteria is rare and has usually a fatal outcome (3, 8, 14, 16, 18, 20, 23, 24, 25, 27, 28, 30, 31, 32). The question which arises in such cases is why the organism usually considered to be of low pathogenicity in man results in a fatal disease. It has been presumed that those patients who develop clinical disease have greater susceptibility. In some cases a primary systemic disease such as leukemia might have been the cause of lowered resistance for infection (18). In the present case *Mycobacterium kansasii* was repeatedly isolated during life and cultured from visceral organs at necropsy. Probably in this case too lowered resistance of the host was an important factor in the pathogenesis. The cause of the diminished resistance did not seem to be another underlying disease although it might have been attributed to a subclinical infection with *Toxoplasma gondii*. We did not succeed however in isolating the organisms on inoculation. Post mortem microscopic examination did not reveal any finding which would indicate the presence of a hematological malignancy or another systemic disease. Nor could the treatment with corticosteroids account for the increased susceptibility to infection. This treatment was instituted only one week before the first isolation of mycobacteria and the patient had clinical signs of an infectious disease for at least two months before. A primary immunological deficiency is unlikely since she had been well before the actual illness.

The clinical symptoms were dominated by fever, loss and occasionally unproductive cough. Granulocytopenia was an early and persistent laboratory finding. Later on thrombocytopenia and marked anemia developed and the pancytopenia

became severe during the last months of her illness requiring blood transfusions. On many occasions aspiration of sternal marrow proved difficult to perform and yielded only a sparse number of marrow particles. However, histological examinations of biopsies from the spine consistently showed a hypercellular marrow devoid of fat and a delicate augmentation of reticulin fibrils. There was however no fibroblastic proliferation of the type seen in primary myelofibrosis. The number of megakaryocytes examined on sections was normal. Examination of bone marrow smears on different occasions showed a relative increase of erythroid cells and a granulocytic hypoplasia. The granulocytopenia was pronounced from the beginning at a time when the spleen was hardly felt on palpation. Treatment with prednisolone had no influence on the granulocyte count. The anemia was due to infection in combination with a hemolytic component. Later on the enlargement of the spleen might have been a factor aggravating the anemia. The alternative diagnosis in this case presenting fever, granulocytopenia and anemia was a malignant granulomatous disease within the reticulo-endothelial organs.

It is well established that active disseminated tuberculosis may be associated with a variety of hematological disorders. There are many reports of tuberculosis associated with leukemoid reactions and leukemia (10, 26), myelofibrosis and polycythemia (1), granulocytopenia, thrombocytopenia and pancytopenia (4, 7, 9). In some cases hypersplenism has been suggested as a mechanism of pancytopenia. In many cases tuberculosis seems to be a complicating infection in a primary hematological disorder; in others, especially in the pancytopenias, disseminated tuberculosis seems to be the primary disease. However, not enough attention has been directed to the association of blood dyscrasias accompanied by disseminated infections with atypical mycobacteria. Hitherto twenty-two cases of disseminated visceral infection with atypical mycobacteria have been reported. Nine of them were due to the photochromogenic group I (3, 14, 20, 30, 32), eleven to the non photochromogenic group III (8, 16, 18, 23, 24, 25, 27, 28, 31) and two to the scotochromogenic group II type of mycobacteria (18, 32). A comparison between the clinical data reported for patients with the group I type of infection on the one hand and the group III type on the other suggests that there

might be differences in the clinical picture of these two groups. Nine of the eleven patients infected with the group III mycobacteria were children while all but one of the nine cases infected with the group I mycobacteria were adults. All patients in both groups in whom blood values were reported had anemia and many had a severe anemia which required blood transfusions. There was however a striking difference as regards the white blood cell count. In the seven cases with group III mycobacterial infection whose white cell count was stated all but one had a pronounced leucocytosis. In four cases the count was above 30 000 associated with a moderate granulocytic immaturity and these were judged to be leukemoid reactions. In one case of group II infection reported by McCusker and Green there was a white cell count of 155 000/mm³ with numerous immature myeloid cells (blasts, promyelocytes and myelocytes) in the peripheral blood. In this case a diagnosis of chronic granulocytic leukemia was made and the patient received cytostatic therapy six months prior to the first suggestion of a mycobacterial infection (18). It is possible that in this case mycobacterial infection was a superposition. At autopsy however no leukemic infiltrates were found in other organs than the bone marrow. On the other hand in the patients infected with the group I mycobacteria six of seven for whom values were reported had a pronounced granulocytopenia. Thrombocytopenia was a finding in three cases of group III infection and in all five cases of group I for whom the platelet count was stated. The platelet count was usually normal on admission but like the present case the count decreased with worsening of the patient's condition (14, 16, 32). The bone marrow was usually cellular or hypercellular in infections due to group III mycobacteria. In the cases infected with group I mycobacteria granulocytic hypoplasia was found in three cases (14, 32), bone marrow sections showed a normal cellularity in three cases (14, 32) and hypocellularity in one case (32). As in the present case aspirated bone marrow smears showed in some cases a hypocellularity but bone marrow sections when examined appeared to be hypercellular (14, 32). Granulomata in the bone marrow in the presence and absence of acid fast bacilli were more often found in group III and group II infections but only in one case infected with the group I mycobacterium. Bone lesions

were also more often reported in disseminated infection with group III mycobacteria and all of them were recorded in children. The skeletal lesions in the children in some cases gave suspicion of reticulo-endothelioses. Miliary infiltrates in the lungs on chest X ray examination were occasionally found in both types of infection but many were normal or uncharacteristic. The tuberculin skin tests were negative in all but one case insofar as these tests have been reported. Skin eruptions have been reported in several cases (16, 28, 32). Skin ulcerations with underlying bone lesions were reported in the group III type of infection (25, 27).

The patho-anatomical findings in the present case showed necrotic lesions in lymph nodes and spleen with little granulomatous reaction as described earlier in generalized disease due to *M. tuberculosis* (2) and photochromogenes (3). A characteristic feature was the presence of numerous large beaded bacteria. The demonstration of such bacteria seems to be the only diagnostic criterion also in chronic granulomatous lesions due to group I mycobacteria as these lesions exhibit all the histopathologic features seen in infections with *M. tuberculosis* (13). The suspicion of a granulomatous disease derived in the present case from a liver biopsy. The diagnosis of group I mycobacteriosis was however due to bacteriological procedures.

Most of the hitherto reported cases of disseminated infection with atypical mycobacteria had a fatal outcome despite therapy. However some although short lived effect of antituberculous therapy could be observed (25, 32). Remissions after effective drug therapy were followed by relapses probably due to the emergence of drug resistance. It has been pointed out (25) that in disseminated infections with poor immune response it is wise to give at least four drugs together especially in the initial phase of treatment. The drugs worth trial include streptomycin, erythromycin, ethambutol and cycloserine. Early diagnosis and laboratory tests of drug efficacy are important.

Hematological dyscrasias and hepato-splenomegaly are common findings in cases of disseminated mycobacterial infection. It should be stressed that disseminated infection with atypical mycobacteria may be the cause of a variety of hematological disorders in the same way as dissemination with *M. tuberculosis*. On the other hand infections with atypical mycobacteria may be super-

imposed on primary hematological disorders and other systemic diseases with lowered resistance of the host

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AN IN VITRO DEMONSTRATION OF CELLULAR IMMUNITY AGAINST AUTOLOGOUS MAMMARY CARCINOMA IN MAN

PRELIMINARY REPORT

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Abstract In five out of eight patients operated on for mammary carcinoma, an extract of the autologous tumour induced inhibition of the *in vitro* leukocyte migration. Extracts of the patient's normal mammary tissue gave no inhibition and negative results were likewise obtained when leukocytes from matched controls were exposed to extracts of isogenic normal or tumorous tissue. These observations suggest that in some patients mammary carcinoma is associated with a state of cellular hypersensitivity against components of the tumour.

Very few data concerning cellular immunity against autologous tumours in humans are available (5). Hughes and Lytton (3) employed intracutaneous injection of extract of autologous tumour using as control extract of corresponding normal tissue from the same patient. They found a positive delayed hypersensitivity response with a negative control in 11 out of 33 patients. 16 had mammary carcinoma, three of whom showed a definite positive response whereas in two a weak positive reaction was obtained.

Hellström et al. (2) demonstrated that the growth of autologous neuroblastoma cells *in vitro* was inhibited by lymphocytes from children who were either cured of neuroblastoma or who had persistent tumours whereas the growth of autologous skin fibroblasts was not inhibited. This selective inhibition was also seen with lymphocytes from other children with neuroblastoma and from four of five mothers whose children had neuroblastoma. Lymphocytes from a variety of control patients including children with other malignant tumours were uniformly negative.

In this communication the preliminary results of an *in vitro* analysis of the response of leuko-

cytes from patients with mammary carcinoma to an extract of their own tumour are reported. The system employed is the leukocyte migration technique (1). Evidence has been presented (6) that in this test system an antigen induced inhibition of the migration is a correlate to cellular (delayed type) hypersensitivity. If mammary carcinoma is associated with a state of cellular hypersensitivity directed towards antigenic tumour components this might be revealed by the technique employed.

MATERIAL AND METHODS

Eight consecutive female patients with mammary carcinoma constitute the clinical material (Table I). None of the patients had clinical evidence of metastases or of local inflammatory reaction. At operation the tumour was excised *in toto*. A portion was taken for histological examination, and the tumour was then separated under aseptic conditions at the same time a part of the glandular tissue which was not macroscopically invaded by the tumour was obtained. These specimens were cut into small pieces suspended in Hanks solution and homogenized in a BSE homogenizer for 5 min. The homogenates were kept at 4°C overnight and then centrifuged at 1000 g for 20 min. The protein concentration of the supernatant was adjusted to 1 mg per ml by dilution with Hanks solution.

Mammary glandular tissue obtained at autopsy from patients without malignant disease was prepared in a similar fashion.

The tumours were classified histologically according to criteria discussed previously (4).

The leukocyte migration test was performed as described by Bendixen and Søborg (1). In pilot experiments the highest concentration of extract not causing unspecific inhibition of migration was 100 µg protein per ml culture medium, and this dose was used throughout.

On the day following mastectomy blood was

Table I Data on eight patients with mammary carcinoma

Pat	Age	Histology of tumour				Migration index					
		Histological type	Grade of anaplasia ^b	Lymphocytic infiltration (0 - + + +)	Necrosis (0 - + + +)	Patient			Control		
						PTu	PGIT _i	AuGIT _i	PTu	PGIT _i	AuGIT _i
1	70	Invasive duct carcinoma	III	(+)	0	0.91	1.21	1.02	1.18	1.13	0.95
2	70	Invasive duct carcinoma	II	(+)	0	0.74	0.86	0.89	0.90	0.91	0.89
3	70	Invasive duct carcinoma	II	0	0	0.63 ^c	1.04	0.95	0.97	1.36	0.97
4	71	Invasive duct carcinoma	II	+	0	0.79 ^c	1.06	0.94	1.02	1.01	1.04
5	46	Partly lobular carcinoma	II	0	0	0.73 ^c	1.05	0.99	0.90	0.97	0.95
6	68	Invasive duct carcinoma	II	+	0	1.10	1.05	1.06	1.12	1.14	1.09
7	45	Invasive duct carcinoma	III	++	+	0.74 ^c	1.00	1.02	1.04	0.94	1.00
8	51	Invasive duct carcinoma	III	+	0	0.97	0.98	0.97	0.94	0.95	0.94

The results of the leukocyte migration test are expressed as Migration index (see text). As antigens were used extracts of patient's mammary tumour (PTu), patient's non tumorous mammary glandular tissue (PGIT_i), mammary tissue obtained at autopsy from patients without malignancy (AuGIT_i).

^a Grading of anaplasia: I low, II medium, III high.

^c Cultures showing inhibition.

heparin. After sedimentation the leukocytes were washed 4 times in Hanks solution and collected in capillary tubes. Each of these was placed in a culture chamber containing 10 ml of TC 199 with 10% horse serum. To the test cultures 0.1 ml tissue extract containing 100 µg protein was added. All cultures were set up in quadruplicate. After 74 hours the migration area of leukocytes around the opening of the capillary tube was measured in a projection microscope. Within a set of identical cultures the variation from one migration to another did not exceed 10%. The average migration area of extract-containing (M_i) and control (M₀) cultures from the same blood sample determines the migration index (MI).

$$MI = \frac{M_i}{M_0}$$

A value of less than 1.0 thus indicates an inhibition of migration. For each patient examined a control patient without malignant disease, matched for age and sex, was studied in parallel series of cultures.

RESULTS

The results obtained in the eight patients and their matched controls are summarized in Table I. In

cultures from the control patients the addition of mammary extract (100 µg protein) from both tumorous and normal tissue gave no inhibition of leukocyte migration; the values for MI ranging from 0.89 to 1.36 with a mean of 1.01. In five of the patients with mammary carcinoma an inhibition of leukocyte migration (MI below 0.80) was induced by the tumour extract. In no instance was inhibition induced by extract of the patient's own non tumorous mammary tissue or by extract of isogenic mammary tissue obtained at autopsy.

DISCUSSION

It is of interest to compare the two *in vitro* techniques by which a state of cellular immunity against autologous malignant tissue has been demonstrated in man: the colony inhibition assay of Hellstrom et al. (2) and the migration inhibition test described in the present report. In colony

inhibition cytotoxicity of sensitized lymphocytes against target cells is demonstrated. With this technique the question of necrotic antigens i.e. acquisition of new antigens solely due to necrosis in the tumour does not arise. This source of error cannot be excluded in the migration inhibition test. On the other hand this technique is open to much wider applications since it does not depend on the availability of living target cells in culture.

The application of such a technically simple in vitro technique for the detection of cellular anti-tumour immunity in man seems to offer great possibilities. A larger series is necessary in order to investigate the relationship between histological appearance and presence or absence of immunity. Also investigations of pre-malignant conditions and non-malignant states with mammary necrosis may prove of interest. Follow-up studies of the individual patients can establish whether a positive immunological response influences the prognosis. The future development of the reaction must be followed both in patients in whom the treatment proves radical and in those who develop metastases. It will be of interest to establish whether the immune reactivity disappears in patients who are overwhelmed by their tumour.

Examination of the specificity of the response will reveal whether patients react exclusively with their own tumour or whether cross-reactions with extracts of other tumours can be demonstrated—the latter possibility corresponding to tumours induced in syngeneic animals by the same virus as opposed to the immunological uniqueness of tumours induced by the same chemical carcinogen.

By means of this technique it seems feasible to characterize the antigens responsible for the reaction with regard both to their subcellular localization and their biochemical nature.

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HAEMODYNAMIC EFFECTS OF TRASICOR® A NEW BETA ADRENERGIC BLOCKING AGENT IN HYPERTENSIVE PATIENTS

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Abstract Five hypertensive patients with left ventricular hypertrophy and minimal pulmonary signs of left-sided heart failure were treated for five weeks with oral Trasacor®, a new beta adrenergic blocking agent. During the course of the treatment the basal arterial pressure in the upright position declined very slightly. Haemodynamic studies were performed at rest and during submaximal supine exercise before and during beta blockade. Negative chronotropic action on the heart was obvious. Whether the drug had any negative or positive inotropic effects on the heart remained uncertain because of inconsistent haemodynamic changes and because of the relatively profound reflex and autoregulatory alterations in left ventricular performance induced by exercise.

Recently several new drugs thought to act as beta adrenergic blocking agents have been developed. Increased interest in finding new compounds of this sort has been due to the probable clinical usefulness of beta adrenergic blockade as well as to the need to develop a drug with minimal depressing effect on myocardial contractility yet with properties of a beta blocker.

Results concerning the antihypertensive effect of the beta adrenergic blocking agents have been inconsistent (3, 5-9, 11-13) and therefore the therapeutic value of these drugs in arterial hypertension is still debatable. In the present paper we report the haemodynamic effects of prolonged peroral administration of a new beta adrenergic blocking agent, Trasacor® in patients suffering from essential hypertension with left ventricular hypertrophy. The haemodynamic studies were done at rest and during submaximal exercise.

MATERIAL AND METHODS

Fifty patients, two females and three males, aged 43-53 years, volunteered for the study. The patients were se-

lected in the outpatient clinic on grounds of radiological and/or electrocardiographic evidence of left ventricular hypertrophy with minimal or absent signs of left ventricular failure. The duration of diagnosed hypertension in the patients was 1-15 years. Their disease was classified as essential hypertension by exclusion of renal and endocrinological causes of hypertension, urinary sediment, urinary protein excretion, endogenous creatinine clearance, intravenous urography, serum sodium, potassium and pH and urinary excretions of vanillylmandelic acid and metanephrines were all normal. Coronary and valvular heart diseases were also excluded.

The patients were admitted to hospital for haemodynamic studies and initiation of the treatment. Previous antihypertensive medication was withheld two weeks before hospitalization and throughout the trial and no drugs were allowed except the one to be tested. During the first week in hospital (the third week without any treatment) the arterial pressure was measured in the upright position every morning with a sphygmomanometer and at the end of the week the first haemodynamic study to obtain the initial values, was performed. Thereafter treatment with Trasacor® (1-*Co-allyloxyphenoxy*) 3-isopropylamino-2-propanol hydrochloride) was initiated (Trasacor® was supplied from Ciba A.G. Basel). The initial dosage given as four divided doses per os, was 160 mg daily for one week. In the 2nd week the dosage was increased to 240 mg daily, in the 3rd week to 320 mg daily and in the 4th week to 400 mg daily. The patients were discharged in the 2nd week of treatment, studied twice a week in the outpatient clinic for possible untoward symptoms or signs, and admitted to hospital at the end of the 4th week of treatment. The daily dosage of 400 mg was continued for another week in hospital and upright arterial pressure was measured daily. At the end of the 5th week of treatment the haemodynamic study was repeated.

The haemodynamic studies were performed similarly before and during treatment with 400 mg Trasacor® daily in the morning and while the patients were in a fasting state. The pressures in the pulmonary artery and in the pulmonary capillary wedged position were measured by right heart catheterization and the pressures in the brachial artery by puncture and cannulation with a teflon catheter. The pressure pulses were recorded utilizing a Sanborn

Table 1 Haemodynamic data at rest and during exercise before and during treatment with Trasacor[®]

	Pat 1		Pat 2		Pat 3		Pat 4		Pat 5	
	Before	During	Before	During	Before	During	Before	During	Before	During
Heart rate (beats/min) at rest	88	70	63	58	98	81	86	78	86	86
during exercise	140	120	105	94	16	132	138	175	147	170
Cardiac output (l/min) at rest	7.4	6.4	6.5	5.0	10.5	4.7	4.0	3.9	6.4	6.0
during exercise	17.5	15.6	11.1	10.1	13.6	8.1	10.2	7.2	13.6	13.8
Stroke volume (ml) at rest	88	91	103	85	106	59	46	50	74	91
during exercise	125	130	106	107	85	61	74	58	96	115
Mean brachial artery pressure (mm Hg) at rest	190	163	140	149	144	156	160	180	160	176
during exercise	160	14	160	157	156	167	185	200	194	208
Systemic vascular resistance (units) at rest	26	25	21	29	13	33	39	45	24	29
during exercise	9	9	14	15	11	22	18	18	14	15
Mean pulmonary capillary wedge pressure (mm Hg) at rest	11	11	8	8	10	5	10	12	8	9
during exercise	14	0	24	24	17	11	11	10	17	17
Mean pulmonary artery pressure (mm Hg) at rest	21	20	16	16	16	17	17	23	16	—
during exercise	—	9	38	34	26	25	—	17	20	—
Pulmonary vascular resistance (units) at rest	14	14	12	16	0.6	7.6	18	28	12	—
during exercise	—	0.6	0.7	1.0	0.7	1.6	—	0.3	0.6	—
LV ejection time (sec) at rest	0.30	0.32	0.27	0.28	0.25	0.23	0.28	0.30	0.26	0.30
during exercise	0.24	0.27	0.23	0.25	0.22	0.23	0.22	0.25	0.22	0.27
LV mean systolic ejection rate (ml/sec) at rest	290	285	380	304	480	236	164	167	290	304
during exercise	5.0	481	460	478	386	66	336	320	436	4.5
LV minute work (kgm/min) at rest	20.2	15.0	13.1	10.7	21.7	10.5	9.7	10.1	14.7	15.2
during exercise	40.1	31.8	25.4	22.8	30.4	71.2	27.1	20.6	37.8	41.1
LV stroke work (kgm) at rest	0.4	0.21	0.21	0.18	0.22	0.13	0.11	0.13	0.17	0.3
during exercise	0.29	0.27	0.24	0.24	0.0	0.16	0.0	0.17	0.27	0.34
Rate pressure product (units) at rest	227	164	126	127	196	16	185	197	172	166
during exercise	30	183	220	183	300	767	274	82	296	311

pressure transducer (model 67B) with optical galvanometers on photographic paper at a speed of 40 mm/sec. First the pressures at rest in the pulmonary artery, pulmonary capillary wedged position and brachial artery, the heart rate and the cardiac output using the Fick prin-

Table 11 Systemic arterial blood pressure before and after 5 weeks' treatment with oral Trasacor[®]

Pat	Arterial pressure in the upright position as measured by sphygmomanometer		Intra-arterial pressure during cardiac catheterization at rest	
	Before	During	Before	During
1	00/100	195/80	70/120	234/11
2	205/110	175/105	200/115	19/116
3	175/115	155/100	00/11	200/120
4	205/115	190/110	15/110	53/140
5	225/130	220/115	200/110	25/134

ciple were determined with the patient in the supine position with his lower extremities already connected to a bicycle ergometer to achieve the same initial position as during exercise. Thereafter exercise was started first with a load of 150 kgm/min for two minutes to get the patient accustomed to the procedure and then with a load of 300 kgm/min which was maintained for at least four minutes, before the same haemodynamic measurements at rest were repeated while the patient was still exercising.

The following variables were calculated from the haemodynamic data: systemic and pulmonary vascular resistance, stroke volume, left ventricular ejection time (1/a mean of five consecutive beats from brachial artery pressure curves), mean systolic ejection rate, left ventricular minute work and stroke work, and the rate pressure product of systemic circulation as defined by R. Hume (10).

The chest radiographs were taken before and during the treatment without reference to the cardiac cycle. The volume of the heart, the relative sizes of its chambers and of the large blood vessels, and the radiological signs of left ventricular failure were estimated by the method described previously from this laboratory (14).

Table III Findings in the chest radiographs before and during treatment with Trasicor®

	Pat	Heart volume		Grade of enlargement		Left ventricular failure
		Absolute (ml)	Relative (ml m ² BSA)	Left atrium	Left ventricle	
Before	1	1000	550	1	1	1a
During		930	540	1	1	1b
Before	2	785	485	1	0	1a
During		1100	670	2	1	1a
Before	3	710	410	1	1	1b
During		650	375	1	1	1b
Before	4	1160	590	2	1	1a
During		1140	580	2	1	2
Before	5	1000	540	1	1	1a
During		1090	575	1	1	1a

Left atrium

0 = normal

1 = slight but no longer normal indentation in the lateral view of the oesophagus

2 = distinct displacement of the oesophagus

Left ventricle

0 = normal

1 = slight rounding of the apex

2 = distinct enlargement and displacement downward or outward

Left ventricular failure

0 = normal

1a = venodilatation in the lower and middle fields and/or slight hilar or pulmonary clouding

1b = marked venodilatation in all the pulmonary fields and/or distinct clouding

2 = venoconstriction in the lower fields attended by marked venodilatation in the upper fields

3 = distinct alveolar pulmonary oedema

RESULTS

The results are given in Tables I II and III and in Fig 1

Heart rate

The treatment with Trasicor® decreased the heart rate at rest as well as the heart rate response to the same work load. At rest the heart rate was 5–20 beats slower (a mean of 15° less than the pretreatment values) and during exercise correspondingly 11–30 beats (mean 13°) slower during treatment than before it. However the relative rise in heart rate in response to the same work load was similar both before and during treatment.

Cardiac output

At rest the cardiac output was always less during treatment than before it, the decline varying between 0.1 and 5.8 l/min (mean 2.0°). During the increase of cardiac output remained during treatment than before it the difference lying between 1.0 and 5.5 l/min except in one patient (pat 5) whose exercising cardiac output

was unchanged (mean decrease 18°). However the relative increase of cardiac output in response to exercise was similar both before and during treatment.

Stroke volume

At rest no constant change in stroke volume was induced by treatment. During exercise the stroke volumes usually increased slightly but no constant difference was found between exercising stroke volumes before and during treatment.

Brachial artery pressure

Arterial pressure calculated as a mean of the sphygmomanometer measurements on seven mornings in hospital before and during treatment showed a decrease in all patients during treatment (Table II). The systolic pressure declined by 5–30 mm Hg (mean 9°) and the diastolic pressure by 15–20 mm Hg, except in one patient whose mean diastolic pressure rose by 5 mm Hg (mean decrease 10°). The direct intra-arterial pressures measured during cardiac catheterization

Table 1 Haemodynamic data at rest and during exercise before and during treatment with Trasicor¹

	Pat 1		Pat 2		Pat 3		Pat 4		Pat 5	
	Before	During	Before	During	Before	During	Before	During	Before	During
Heart rate (beats/min) at rest	111	70	111	58	98	81	86	78	111	111
during exercise	140	110	105	94	16	132	138	125	147	170
Cardiac output (l/min) at rest	7.4	6.4	6.5	5.0	10.5	4.7	4.0	3.9	6.4	6.0
during exercise	17.5	15.6	11.1	10.1	13.6	8.1	10.2	7.2	13.6	13.8
Stroke volume (ml) at rest	111	91	103	85	106	59	46	50	74	91
during exercise	125	130	106	107	111	61	74	58	96	115
Mean brachial artery pressure (mm Hg) at rest	190	163	140	149	144	156	160	180	160	176
during exercise	160	142	160	157	156	187	185	200	194	201
Systemic vascular resistance (units) at rest	26	25	21	29	13	33	39	45	24	29
during exercise	9	9	14	15	11	27	18	28	14	15
Mean pulmonary capillary wedge pressure (mm Hg) at rest	11	11	8	8	10	5	10	12	8	9
during exercise	14	20	24	24	17	11	11	10	12	12
Mean pulmonary artery pressure (mm Hg) at rest	21	20	16	16	16	17	17	23	16	—
during exercise	—	29	28	34	26	25	—	1	0	—
Pulmonary vascular resistance (units) at rest	1.4	1.4	1.2	1.6	0.6	2.6	1.8	2.8	1.2	—
during exercise	—	0.6	0.7	1.0	0.7	1.6	—	0.3	0.6	—
LV ejection time (sec) at rest	0.30	0.32	0.27	0.28	0.25	0.25	0.28	0.30	0.6	0.30
during exercise	0.24	0.27	0.23	0.25	0.22	0.23	0.22	0.25	0.24	0.27
LV mean systolic ejection rate (ml/sec) at rest	290	295	380	304	480	236	164	167	290	304
during exercise	520	481	460	428	386	266	336	232	436	425
LV minute work (lpm/min) at rest	20.2	15.0	13.1	10.7	21.7	10.5	9.2	10.1	14.7	15.1
during exercise	40.1	31.8	25.4	27.8	30.4	21.2	27.1	20.6	37.8	41.1
LV stroke work (kpm) at rest	0.24	0.21	0.21	0.18	0.22	0.13	0.11	0.13	0.17	0.23
during exercise	0.29	0.27	0.24	0.24	0.20	0.16	0.20	0.17	0.27	0.34
Rate pressure product (units) at rest	77	164	126	127	196	162	185	197	177	166
during exercise	30	188	20	183	300	267	274	282	296	311

pressure transducer (model 67B) with optical galvanometers on photographic paper at a speed of 40 mm/sec. First the pressures at rest in the pulmonary artery, pulmonary capillary wedged position and brachial artery, the heart rate and the cardiac output using the Fick prin-

ciple were determined with the patient in the supine position, with his lower extremities already connected to the bicycle ergometer to achieve the same initial position during exercise. Thereafter exercise was started first with a load of 150 kpm/min for two minutes to get the patient accustomed to the procedure, and then with a load of 300 kpm/min which was maintained for at least 5 minutes, before the same haemodynamic measurements at rest were repeated while the patient was still exercising.

The following variables were calculated from the haemodynamic data: systemic and pulmonary vascular resistance, stroke volume, left ventricular ejection time (a mean of five consecutive beats from brachial artery pressure curves), mean systolic ejection rate, left ventricular minute work and stroke work, and the rate pressure product of systemic circulation as defined by Robinson (10).

The chest radiographs were taken before and during treatment without reference to the cardiac cycle. The volume of the heart, the relative sizes of its chambers and of the large blood vessels, and the radiological signs of left ventricular failure were estimated by the methods described previously from this laboratory (4).

Table II Systemic arterial blood pressure before and after 5 weeks' treatment with oral Trasicor¹

Pat.	Arterial pressure in the upright position as measured by sphygmomanometer		Intra-arterial pressure during cardiac catheterization at rest	
	Before	During	Before	During
1	200/100	195/80	201/0	234/112
2	205/170	175/105	200/115	219/116
3	175/115	155/100	200/117	200/170
4	220/125	190/130	215/10	253/140
5	224/170	220/115	200/130	252/134

Mean pulmonary capillary wedge pressure

No significant change in the resting mean PCW pressures was induced by the treatment. During exercise without treatment the mean PCW pressure increased in all by 1–16 mm Hg. During treatment the pressures increased to the same extent as without treatment in individual patients. One patient (pat. 2) showed a mean exercising PCW pressure of 24 mm Hg both before and during treatment.

Pulmonary artery mean pressure and vascular resistance

No consistent changes were observed in the cases in which measurements were performed.

Left ventricular ejection time

At rest the left ventricular ejection time increased in four patients and remained the same in one patient roughly corresponding to the changes in heart rate during treatment. During exercise the ejection time was similarly always longer during treatment than before it (mean increase 13%). The change could be ascribed to the slower exercising heart rate during treatment. The relative mean shortening of the exercising ejection time was slightly less during treatment (12%) than before it (17%).

Mean rate of left ventricular ejection

At rest no constant change in the mean rate of left ventricular ejection was observed during treatment. During exercise the mean ejection rate was always less during treatment than before it, the difference varying from 11 to 120 ml/sec (mean 16%). The relative mean increase of the mean exercising ejection rate was slightly less during treatment (40%) than before it (47%).

Left ventricular work indices

Left ventricular minute work and stroke work at rest did not show constant changes during treatment: a decrease was observed in three patients and a slight increase in two others. During exercise the left ventricular minute work was less during treatment than before it in all but one patient (mean decrease 15%). Exercising stroke work did not change in a constant pattern during treatment. The rate pressure product, which it has been suggested reflects internal left ventricular work (10)

seemed to change in a random pattern both at rest and while exercising during treatment.

Radiological findings

The heart volume remained the same in all but one patient (pat. 2) whose relative heart volume increased from 485 to 670 ml/m² BSA. Radiological pulmonary signs of left ventricular failure progressed in another patient (pat. 4). In the other patients no changes in radiological signs were seen.

Side-effects of the drug

No annoying symptoms or signs of toxicity were observed during the treatment.

DISCUSSION

The antihypertensive effect of five weeks peroral treatment with the new beta adrenergic blocking agent, Trasicor[®] is slight, according to the present results. The systemic arterial pressure in the upright position declined in all the patients during treatment but not to recommendable normotensive levels. However, direct intra arterial measurements during cardiac catheterization showed an increase rather than a decrease of arterial pressure during treatment. This discrepancy may reasonably be explained by the combined effect of supine position at catheterization, the casual nature of the intra arterial measurement, and the psychic stress of the catheterization procedure. The information in the recent literature on the immediate hypotensive effects of intravenously administered beta adrenergic blocking agent is not for the most part comparable with data such as ours obtained during prolonged peroral treatment. At present this beta adrenergic blocking agent does not seem to have much practical value in the treatment of essential arterial hypertension.

The negative chronotropic effect of Trasicor[®] on the resting heart rate and on the response of the heart rate to exercise was obvious.

During the treatment the cardiac output at rest and while exercising was somewhat less than without the drug, suggesting a negative inotropic action or simply a secondary influence on the heart rate response. In fact the stroke volumes and the pulmonary capillary wedge pressures at rest and during exercise remained the same despite the drug, indicating that the left ventricle was capable of the same stroke volume with the same filling

pressure while the heart volumes remained unchanged radiologically. Thus no remarkable depression of myocardial contractility had occurred. With the methodical errors involved the changes of left ventricular ejection times and mean rates of left ventricular ejection while exercising during treatment do not seem significantly different from the pretreatment values. Left ventricular minute work paralleled the cardiac output during treatment and stroke work paralleled the stroke volumes as no remarkable changes in systemic vascular resistance occurred. Thus none of our haemodynamic findings show a definite negative or positive inotropic effect of Trasicor®.

On the other hand it is worth noticing that in the intact circulation even with increased left ventricular afterload and myocardial hypertrophy such as our patients had the immediate reflex and autoregulatory adjustments of myocardial contractility induced by exercise may well mask any negative inotropic effects of a drug especially in the supine position. (2) The most important of these regulatory factors influencing the normal cardiac response to exercise are increase in heart rate, sympathetic drive and operation of the Frank-Starling mechanism each of which may also operate independently according to the degree of load applied. (1) During supine submaximal exercise the requirements of tissue perfusion are satisfied mainly by an increase in the heart rate provided the heart is not working on the declining part of the ventricular function curve.

Since the introduction of the beta-adrenergic blocking agents caution has been practised in their use in heart failure because of their negative inotropic effects. In the present study no definite haemodynamic signs of impaired performance of hypertrophic left ventricles were observed during prolonged treatment with Trasicor® at rest or during submaximal supine exercise. It seems likely that in daily doses of up to 400 mg orally Trasicor® has no definite injurious effect on the function of a competent hypertrophic left ventricle.

ACKNOWLEDGEMENT

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ADAMS-STOKES SEIZURES IN PATIENTS WITH ATTACKS OF BOTH TACHY- AND BRADYCARDIA A THERAPEUTICAL CHALLENGE

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Abstract The case histories of six patients with Adams-Stokes seizures and attacks of tachy- and bradycardia are reported. Two patients were managed by pacemaker implantation alone while three patients had to be treated with the combination of pacemaker and a beta receptor blocking drug. The necessity of continuous ECG supervision for diagnosis of the arrhythmias, for selection of proper therapy and control during therapeutical trials is stressed.

The introduction of tape recording in clinical electrocardiography and the establishment of more or less automatic methods for rapid analysis of the ECG recorded on tape have made it possible to follow changes in heart rhythm during extended periods of time. Such techniques have already proved helpful in elucidating problems concerning the clinical importance of paroxysmal arrhythmias and the causal relation between such arrhythmias and sudden spells of dizziness and fainting. It is the purpose of this paper to draw attention to a small group of patients complaining of episodes of both palpitation and fainting in whom long term ECG supervision revealed paroxysms not only of tachycardia but also of asystole caused by transitory atrio-ventricular or sino-atrial block. From a therapeutical point of view these patients present a real tricky problem as an effective drug treatment of their tachycardias may increase their tendency to develop conduction disturbances whereas an effective drug prophylaxis of their episodes of block may result in an aggravation of their tendency to develop tachycardias and may be ventricular fibrillation.

MATERIAL

This series comprises six patients, two men and four women aged 51-69 years (Table I). Three of these patients

showed definite signs of coronary artery disease either in their histories or by changes in their ECG and one had rheumatic heart disease whereas the etiology of the heart disease remained obscure in the last two patients. All six patients had had attacks of palpitation and dizziness or fainting for lengthy periods, i.e. from three months to twenty years.

CASE REPORTS

Case 1

Female born 1916 She was admitted to our department in 1964. For two years she had had increasingly severe attacks of tachycardia often provoked by exercise. In between she also had dizzy spells. Various antiarrhythmic drugs had been tried without any success. Her ECG at rest showed a sinus bradycardia at about 40/min (Fig. 1). A bicycle ergometer test (400 kpm for 1 min, 600 kpm for 2 min) provoked ventricular ectopics which progressed to ventricular tachycardia (Fig. 1). Long-term ECG recording also revealed periods of sino-auricular block, sometimes causing fainting, whereas fainting during an attack of tachycardia was rare. Apart from a moderately enlarged heart (720 ml/m²) and the arrhythmias mentioned, no other signs of cardiac disease were found. The patient's functional capacity was severely restricted.

In December 1964 treatment with propranolol was started. The tachycardia was well controlled, but the dizzy spells became more frequent. So the dosage was reduced from 1.0 mg per day to 70 mg per day. In March 1966 the tachycardia reappeared, alternating with sino-auricular block or extreme bradycardia and often causing dizziness or fainting (Fig. 1). Discontinuation of propranolol for one month did not alter the situation. Treatment with procainamide 1 g per day, diphenylhydantoin 300 mg per day and diphenylhydantoin in combination with atropine did not prevent attacks of tachycardia.

It was concluded that propranolol should be given in bigger dosages. However anticipating an aggravation of the spontaneously occurring sino-auricular block which already troubled the patient very much, we decided to implant a fixed rate epicardial pacemaker first. This effectively controlled the bradycardia, and propranolol 1.0 mg per day prevented tachycardia even during exercise.

Table 1 Age, sex and type of heart disease in six patients with alternating tachycardia and block

Case no	Year of birth	Age	Sex	Type of heart disease
1	1916	57	♀	Unknown
2	1913	61	♂	Ischemic
3	1917	56	♂	Rheumatic
4	1905	63	♂	Unknown
5	1907	66	♀	Ischemic
6	1899	69	♀	Ischemic

(This course of events has been described in an earlier publication (6).)

The patient was seen again in March 1968 i.e. two years after pacemaker implantation. She had had no attacks of tachycardia or fainting but complained of palpitations especially during exercise. This turned out to be due to interference between the patient's own heart action and the artificial pacemaker. For this reason the fixed rate pacemaker was replaced by a demand pacemaker. Since then the patient has been fit and well on treatment with propranolol 90 mg per day. During a recently performed exercise test there was no ectopic activity whatsoever (Fig. 3).

Case

Male born 1914. This man was admitted to a local hospital on November 19 1967 because of acute myocardial infarction of the anterior wall. On the second day in hospital he got an embolus in his left femoral artery. The course was also complicated by long lasting hypotension. In periods he had a rather pronounced ectopic activity. After about three weeks he started to get recurrent attacks of tachycardia and fainting. This was the reason for his being transferred to our coronary unit on December 16 1967. On arrival he was in sinus rhythm. The ECG showed signs of recent anterior wall infarction and a few supraventricular ectopic beats. On the first night he suddenly developed ventricular tachycardia which abruptly changed into asystole of 14 sec duration before sinus rhythm recurred (Fig. 4). Antiarrhythmic treatment seemed necessary but because of the asystole occurring during his attack of tachycardia a pacemaker catheter was first introduced into his right

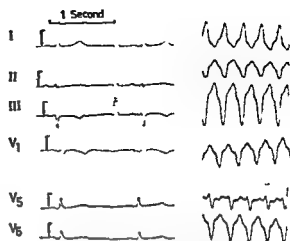


Fig. 1 ECG of case 1 during her first admission in 1964. Left: sinus bradycardia at rest. Right: ventricular tachycardia immediately after a bicycle ergometer test (200 kpm for 1 min, 600 kpm for 2 min).

ventricle. During positioning of the catheter he had several attacks of ventricular tachycardia and fibrillation which had to be stopped by DC shock. Treatment with propranolol in increasing dosages (up to 100 mg per day) was initiated. During the next week no further arrhythmias occurred and the treatment was discontinued. During the next two weeks he had a varying number of extrasystoles (up to 20%) most of which were supraventricular. Besides he had short lasting attacks of tachycardia passing on to bradycardia before sinus rhythm was restored. On January 7 1968 he had cardiac arrest but was easily resuscitated. An ECG was not obtained as monitors had been interrupted. On the next day he had an attack of tachycardia which terminated in asystole during which he fainted. Sinus rhythm recurred spontaneously. Propranolol was started again in a dosage of 80 mg per day. The attacks of tachycardia continued. As they very often terminated in asystole causing fainting it was considered necessary to implant a pacemaker before the dosage of propranolol could be increased. So a transvenous demand pacemaker was inserted on January 12 and the dosage of propranolol subsequently increased to 100 mg per day. This did not abolish tachycardia completely but the fre-

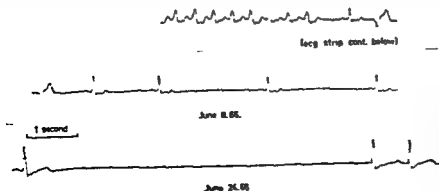


Fig. 2 Bipolar chest lead of case 1. Two upper strips (continuous, June 8 1968): tachycardia terminating in sinus bradycardia. Lower strip (June 26 1968): sinus bradycardia.

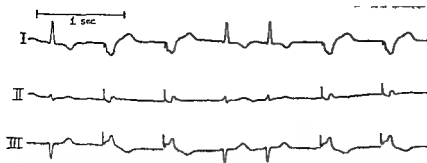


Fig 3 Standard leads I II and III of case 1 showing ECG after a bicycle ergometer test, July 1968 (200 kpm for 5 min 400 kpm for 5 min 600 kpm for 2 min.) Treatment propranolol 90 mg per day

quency of attacks was considerably reduced. The pacemaker effectively prevented fainting. The patient was discharged on February 17. His chest X ray still showed an enlarged heart (760 ml/m). His blood pressure was 100/80. There were no signs of heart failure but he was extremely tired and severely incapacitated by intermittent claudication in his left leg. He was seen in the outpatient clinic on March 27, 1968, when he reported two short lasting attacks of tachycardia. Otherwise his condition was unchanged. He died suddenly in his home on April 12, 1968. An autopsy was not performed.

Case 3

Female, born 1912. This woman has rheumatic mitral valvular disease for which a successful closed mitral valvulotomy was done in 1956. A slight diabetes is well controlled with an oral antidiabetic drug.

She was first seen in this department in 1958 because of palpitations. At that time her ECG showed a rather pronounced sinus arrhythmia and some atrial extrasystoles. She was digitalized and remained well till 1963 when she started to have attacks of tachycardia. For this

reason she was readmitted in March 1964. ECG recording during the attacks showed an atrial tachycardia. This was not due to overdigitalization. In between she had sino-auricular block causing dizzy spells and occasional fainting. Because of a slight heart failure she was given a thiazide diuretic. Antiarrhythmic treatment with a long acting quinidine preparation (Kandin-duretter® 600 mg per day) was started and seemed to have some effect in the beginning. However the attacks of tachycardia gradually became more frequent and long lasting. For this reason quinidine was stopped and in 1965 propranolol was given instead in doses up to 80 mg per day. During this treatment the dizzy spells grew more frequent and she had several syncope. Addition of atropine 2 mg per day and reduction of the dose of propranolol did not change the situation. If treatment with propranolol was to be continued it was considered necessary to implant a pacemaker first in order to prevent fainting due to prolonged systole. However the patient was reluctant to receive this kind of treatment. So propranolol was stopped in September 1967. The patient went on having dizzy spells, fainting fits and frequent attacks of tachycardia.

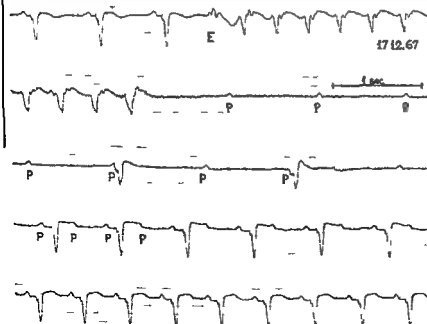


Fig 4 Bipolar chest lead of case 2, December 17, 1967. 1st strip initiation of tachycardia by ectopic beat (E). 2nd strip 3 min 45 sec later abrupt change to atrial asystole which lasted for 14 sec. 3rd strip complete a-v block. 4th strip 2:1 a-v block. 5th strip sinus tachycardia 10 min after onset of the attack.

Table II *Type of tachycardia and block in six patients complaining of palpitation and spells of dizziness or fainting*

Case no	Tachycardia		Block		Tachycardia terminating abruptly in block
	Supra ventr	Ventr	Sino auric	Atrio ventr	
1	-	+	+	-	-
2	-	+	-	+	+
3	+	-	+	-	+
4	+	-	+	-	+
5	+	-	+	-	+
6	+	+	+	-	+

s arrhythmia which caused syncope

implantation and propranolol Patient no 4 has had a demand pacemaker implanted but as a stable position of the catheter electrode has not been obtained antiarrhythmic drug treatment has not yet been started

DISCUSSION

To our knowledge patients of this kind presenting with complaints of both palpitation and dizziness or fainting and the electrocardiographic findings of tachycardia alternating with block have not been mentioned very often in the literature

Cohen et al (1) have reported the successful use of a pacemaker in a patient with supraventricular tachycardia alternating with sinus and nodal bradycardia They suggest that establishment of retrograde ventriculo atrial conduction prevented recurrence of the tachycardia In patient no 5 in this series in whom retrograde atrial activation also occurred during pacing of the ventricles a similar mechanism might have prevented atrial flutter thus offering a possible explanation why this arrhythmia recurred at the time of battery failure

A series of patients with attacks of supraventricular tachycardia and block in whom the com

Table III *Kind of treatment in six patients with alternation of tachycardia and block*

Treatment	No of pati	Cases no
Pacemaker only	3	(4) ^a 5 6
Antiarrhythmic drug + pacemaker	3	1 2 3

^a For reasons given in this patient's case history he is not yet on treatment with an antiarrhythmic drug

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ination of an antiarrhythmic drug and a pace maker was used with success has been reported to us by Sowton (5)

The conclusion of our experience with this small series is that patients complaining of both dizziness or fainting and palpitation should be investigated meticulously by long term ECG recording as their fits of fainting may be caused either by tachycardia or block or both Some of the patients might be relieved by means of drug treatment and others by implantation of a pacemaker whereas a combined treatment with antiarrhythmic drugs and pacemaker implantation may be necessary in several cases According to our experience it seems difficult to predict which kind of treatment will turn out most advantageous Therapeutical trials during continuous ECG supervision are warranted in such patients Before treatment with an antiarrhythmic drug is started we would advise the transvenous insertion of an interim catheter pace maker ready for immediate use in case asystole should be provoked by the antiarrhythmic drug as these patients are predisposed to develop heart block and asystole When permanent pacing is indicated it should be considered in each case which type of pacing—atrial or ventricular demand or fixed rate—should be preferred Atrial pacing which hemodynamically is superior to ventricular pacing (3) seems well indicated in patients without atrial tachycardia fibrillation or atrio ventricular block Demand pacing is suitable when the purpose is to prevent syncope caused by bradycardia or block whereas fixed rate pacing might be preferable when the aim is suppression of tachycardias (2, 4)

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HEREDITARY HEPATIC PORPHYRIAS

Gene Penetration Drug Sensitivity and Subdivision in the Light of Systematic Family Studies

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Abstract On the basis of systematic family investigations of Danish porphyrina patients through ten years with recording in a card index, the gene penetration has been studied in different families. The suggestion of Waldenstrom that gene penetration might vary was confirmed as some families had many manifest cases and a high number of latent cases excreting high concentrations of metabolites while others had only one manifest case and few latent excretors with low metabolite concentration. Further an intermediate status of different degrees exists as illustrated by Figs 1-6. It is concluded that the large families with acute porphyrina in Northern Sweden and with variegate porphyrina in South Africa constitute extremes, the most frequent finding in other countries being families with considerably less pronounced gene penetration. Observations further suggest that the sensitivity to drugs—barbiturates etc.—as provocators of porphyric attacks varies with the gene penetration prevailing in the family, thus the risk of drug provoked attacks seems to be much higher in Sweden and South Africa than in Denmark. It is therefore an important point in the prophylaxis of the porphyric attack to study the family of every patient to get an impression of the gene penetration and drug sensitivity prevailing in that particular porphyric family. It is recommended that a complete porphyrinological investigation be performed in all porphyric patients as well as their close relatives—parents, siblings, children—i.e. screening tests for porphyrin in urine and faeces supplemented by quantitative analyses if positive and—last but not least—ion exchange chromatographic analysis for both porphobilinogen (PBG) and delta aminolevulinic acid (ALA).

This should be done not only in acute intermittent porphyrina (AIP) and variegate porphyrina (VP) but also in hereditary coproporphyrina (HCP) and hereditary porphyrina cutanea tarda (PCT II). Examples from the Danish material are given. The findings indicate that the subdivisions between different forms of hereditary hepatic porphyrias are more or less artificial as really every family constitutes its own form of porphyrina. Even a rare form such as HCP varies profoundly from one family to another. The diagnostic values of systematic family investigations is exemplified and stressed.

While porphyrins are well defined compounds the concept of porphyrina is far more floating. Actually every investigator actively engaged in porphyrina research has his own version of the definition and subdivision of this group of diseases. For that of the writer the reader is referred to his recent survey on the clinical chemistry of porphyrias (27). It therefore seems evident that the subdivision and nomenclature of porphyrias can only be provisional and tentative and do not constitute a firmly established system.

During his study of porphyrias in Denmark for over 20 years a study which has included systematic family investigations with chemical tests for ten years the writer (23-25) became increasingly intrigued by the difference between his findings in Denmark and those of Waldenstrom and his group in Sweden (18, 19, 22). Simultaneously the Waldenstrom group became similarly intrigued by the differences between their own findings and those of the South African workers concerning variegate porphyrina (7).

A way of explaining these unquestionably real differences between the observations on different forms of hereditary hepatic porphyrias in different countries was pointed out by Waldenstrom and Haeger-Aronsen (19) who suggested that the gene penetration in the big family with acute intermittent porphyrina (AIP) in Northern Sweden was exceptionally high. It seemed natural to go further and suggest that the gene penetration in the large South African family with variegate porphyrina (VP) was correspondingly high as this would explain the differences observed between VP in South Africa and in other countries. Further

observations suggest that drug sensitivity may be related to gene penetration because the Swedish and South African experiences of the risk of latent porphyrics taking barbiturates contrast clearly with Danish observations. Finally our own studies of a family in central Jutland with hereditary coproporphyrin (11) suggest that this disease cannot really be an entity but must consist of a group of diseases with varying clinical and chemical features and severity.

The author therefore believes that every porphyric family has its own characteristic disease which has once originated as a mutation perhaps in one locus characteristic of the hepatic porphyrias perhaps in different loci close to each other. Consequently every family should constitute a special entity as regards symptoms, gene penetration and sensitivity to pregnancy and drugs—and the evidence from the writer's record of Danish porphyria families was found to give some support to this view. This hypothesis does not contradict Watson's (20) concept of hepatic porphyria as a melting pot, our concept of a series of families with different diseases being based on our systematic family studies which are possible in Denmark, while similar studies can hardly be successfully performed in most other countries.

METHODS

The analytical methods employed have been described by With (3, 27). The morning urine of every patient and relative was subjected to ion exchange chromatography, determination of porphobilinogen (PBG) and delta aminolaevulinic acid (ALA) as well as to screening of porphyrins; the faeces were screened for porphyrins. Quantitative analysis was performed if screening was positive. The patients from the last two years were further studied with thin layer chromatography of extracts of their excreta by the methods introduced by the writer employing talc layers (26, 28).

Family investigation was offered to every patient. This proceeded as follows. First the patient or one of the patient's relatives constructed a table of the close relatives (parents, siblings, children) with names and addresses. Then mimeographed standard letters were sent from our laboratory to the relatives, accompanied by material for collection of urine and faeces. The letters contained a short account of the porphyria disease in their relative and the risk involved as well as the advantage of knowing beforehand one's eventual state as porphyria carrier. It was made clear that all analyses are carried out without cost to the relatives as a scientific service. On average about 90% of the relatives responded by sending the materials requested. A substantial number of the relatives not responding to the letters were as a rule contacted

later through cooperative members of the family. When the analytical data were ready both the relatives and their physicians received standard letters explaining the implication of being a carrier, should this prove to be the result of the analysis, or explaining that the analyses exhibited normal results on this occasion, which does not with certainty exclude carrier status. The letters also mentioned the drugs which have to be avoided and further stressed the importance of informing all physicians and hospitals with whom the relative comes into contact about their certain or possible state as a porphyria carrier.

In the long run as a rule 30–70% of the relatives were subjected to study in this way but in some families the coverage approached total, because we had the opportunity to contact the subjects several times when new episodes of porphyria developed in their families. The continued efforts through the last ten years have thus resulted in a rather good coverage of several Danish porphyric families but the material is still far from complete although the interest of Danish hospitals and physicians in our work is increasing.

RESULTS

Our findings are illustrated by typical examples from the Danish porphyria register in Figs 1–6. Further examples will be found in With (23). Thiele and With (16) as well as for hereditary coproporphyrin (HCP) in Lombolt and With (11). In Figs 7–9 examples of non porphyric families studied by the same technique are presented as controls. Further, our laboratory possesses a large series of analyses for PBG and ALA in urine as well as porphyrin screening in urine and faeces by the methods mentioned above according to which below 1% of normal adult subjects have ALA above 0.60 and PBG above 0.30 mg per 100 ml of urine or positive screening tests in urine or faeces for porphyrins. This material has been partially published (27) but is continually expanding and at the moment comprises more than a thousand subjects.

In the figures the letter A designates the first generation, B the second etc. Numbers following the letters refer to the sequence in the sibships. Thus A 7 is number seven in the first generation, B 23 is number 23 among the offspring of A 2, C 513 is the third child of B 51 etc. The sex of the subjects is indicated by symbols under the numbers. If the sex symbol is placed within brackets it means that the excreta of the subject in question have not been subjected to chemical study. If the sex symbol is without brackets and underlining the excreta have been studied and found normal if the sex symbol is underlined.

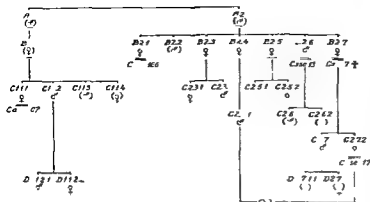


Fig 1 Family (4 generations) with 27 members, 5 cases of porphyria one (case 7) died during a porphyric attack. A1 A2 and B11 are registered as carriers because of cases of porphyria among their descendants. Seventeen family members were studied. 5 patients, one carrier (B25) and 11 with normal excretion. B25 had PBG 50 ALA 0.67 mg/100 ml and normal faeces. C241 and C272 (case 117) are married but without offspring yet.

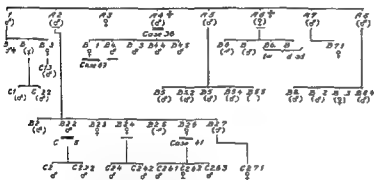


Fig 2 Family (3 generations) comprising 48 members. 4 cases of AIP and 6 carriers. 17 subjects were studied. Case 36 died from porphyria before the family investigation and, together with his daughter (case 69) was reported on by Budolfson et al (*Acta psychiat. scand. suppl. 108* 71 1956). Cases 51 and 141 are both extremely severe and protracted, the former having extensive

residual pareses 11 years after the first attack. All the subjects studied had normal faecal porphyrin. The figures for PBG and ALA (mg/100 ml) were as follows: B24 PBG normal ALA 0.80 B42 1.40 2.00 C242 normal 0.70 C262 3.1 0.3. A. and A4 were registered as carriers because of the cases of AIP among their offspring.

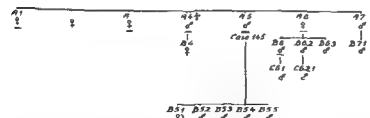


Fig 3 Family (3 generations) comprising 110 members, of whom 17 were studied: one patient and 7 carriers. The faeces of A5 (case 145) showed coproporphyrin 340 and protoporphyrin 135 μ g per g dry matter during the attack. The faeces were normal in all the relatives studied except A1 who showed coproporphyrin 137 and protoporphyrin 37 μ g per g dry matter. The PBG and ALA values were: A1 PBG 2.0 ALA 1.30 A3 0.72, 0.65 A4 3.10 1.00 A5 (case 145) 2.60 1.50 A6 1.25 0.6 B61

117 4.30 A4 later developed a vascular cerebral affection with pareses at that time PBG was 2.60-6.40 and ALA 1.80-4.50. If he had not been known as a latent porphyric an erroneous diagnosis of porphyria as cause of his pareses might have been made. This family shows features of both AIP and VP (mixed porphyria). It is remarkable that the carrier with the highest urinary excretion had normal faecal porphyrin.

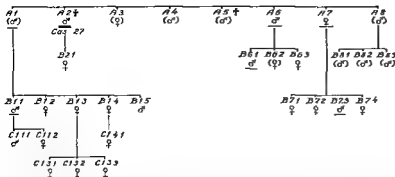


Fig 4 Family (3 generations) with 30 members 20 were studied and only 3 showed increased excretion and only of ALA. The figures were B11 0.70 B61 0.62 and B73 0.62 mg/100 ml. As our normal material comprising more than 1000 cases exhibits ALA above 0.60/100 ml in about 1 of the cases this finding strongly suggests porphyria. The patient (case 27) was a Danish physician

working in a remote part of the Faroe Islands who died from a protracted attack diagnosed as AIP. Chemical verification was not possible. The daughter of the patient (B21) was suspected of AIP but showed normal excretion. Faecal porphyrins were normal in all the subjects. The family is regarded as one of AIP with low gene penetration.

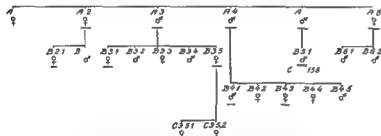


Fig 5 Family (3 generations) with 23 members who were all studied. One patient (B51, case 156) and 13 carriers. A1, A3 and A4 had normal excretion and were registered carriers for genetical reasons. Faecal porphyrins were normal throughout. The PBG and ALA figures were: A5 PBG 0.39 ALA 0.26 A6 0.37 0.24 B21 0.30 0.83 B31 0.38 0.39 B32 0.20 0.69 B35 1.00 0.46 B41 0.22 0.63 B43 0.47 0.61 C351 0.31 0.79 C352 0.47 0.61 mg per 100 ml urine. These values are low but nevertheless definitely abnormal. The patient suffered from polyradiculitis beginning with abdominal pain and later complicated with respiratory and cardiac arrest from which he recovered. His excreta were studied and found normal twice during the later course when he was still paretic. PBG and ALA were not measured during the acute stage but a slight elevation of porphyrins was found in his urine at that time. This case from Copenhagen City Hospital Surgical Department I was studied in collaboration with S. A. Damgaard Nielsen, M.D.

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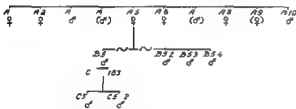


Fig 6 Family with 17 members. 14 were studied and all found to be normal except the patient (B51, case 163), an illegitimate child and his two sons. The patient's father was not available for study. The patient was admitted to the Neurological Department, County Hospital Holstebro, Jutland (Head P. Berggren, M.D.) for polyradiculitis and showed normal PBG and slightly increased ALA during the paralytic stage (PBG 0.30 and ALA 0.70 mg) and normal faecal porphyrins. His sons both showed normal faecal porphyrins and normal PBG but elevated ALA: C511 0.76 and C512 1.10 mg per 100 ml.

once the excreta have been found to contain definitely abnormal quantities of porphyrins and/or precursors on at least one occasion or the subject in question has to be regarded as a carrier because of proved carriers among the offspring. If the sex symbol is doubly underlined it means that the subject has had at least one episode of clinical manifest porphyria, either abdominal neuropathy or cutaneous.

Individual data are found in the explanations to the figures.

DISCUSSION

As seen from Figs 1-6 and the figures of With (23) some porphyric families have several manifest cases—each with its own number in the Dan

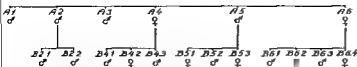


Fig 7 Normal family exhibiting negative values through out in study of 18 members (two generations) All living family members were studied The study was performed

because A2 was suspected of AIP (recurrent attacks of abdominal pain)

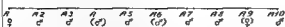


Fig 8 Family consisting of 10 siblings of whom 7 were subjected to study because one (A2) was suspected of AIP (recurrent attacks of abdominal pain). All exhibited a negative urine and faecal screening for porphyrins PBG below 0.30 and ALA below 0.00 mg per 100 ml



Fig 9 Study of the four descendants of a patient with lead intoxication (A 1). The patient showed ALA 0.76 and 0.67 mg per 100 ml on two occasions. All the five subjects showed negative urine and faecal screening for porphyrins and PBG below 0.30 mg per 100 ml. The four children all had ALA below 0.60 mg per 100 ml.

had been mentioned by Waldenström and Haeger Aronsen (19) who suggested that the gene penetration in the big Swedish AIP family might be exceptionally high. We here extend this hypothesis further by assuming a variation in gene penetration from family to family as exemplified in our Figures 1 and 2 being examples of relatively high gene penetration although considerably lower than found in the big Swedish AIP family and Figs 3-6 exemplifying families with lower gene penetration. Our hypothesis includes the concept that negative PBG but positive ALA as well as intermittent excretion and relatively low concentrations in carriers indicates a low gene penetration while the opposite findings indicate a high one. Further we came to the conclusion that the risk for the carriers of developing porphyric attacks because of drug administration—especially barbiturates—most likely varies in parallel with the gene penetration. It was from Sweden that the risk of barbiturates was originally pointed out but in Denmark the carrier state is so widely distributed and barbiturate consumption so common that many porphyrins must have taken barbiturates several times and as porphyric attacks are rare barbiturate sensitivity cannot be high. Add to this the porphyric epileptics who have taken large doses of barbiturate for long times (9, 10). Finally the first case of a porphyric attack published in Denmark (12) received 500 mg of diemal twice and later 100 mg of phenemal as daily injection for 16 days during his illness. His porphyrina did not get worse through this treatment but recovery began immediately after it was stopped. This is not mentioned in the publication but was only recently found out during the author's study of the original case record (from A. W. Sørensen, M.D., Ålborg County Hospital, Ålborg) for at the time of the publication the role of barbiturates in producing porphyric attacks was unknown. According to Swedish and South African experience this patient should be long dead but he developed only a moderately severe attack with

limited pareses of the arms abdominal pains and mental confusion. Also in another of the early Danish cases—no. 26 from Svendborg County Hospital—where the writer recently had the opportunity of studying the original hospital records recovery from an attack of acute porphyria took place in spite of continued administration of 90 mg of phenemal daily and 100 mg of allypropymal as hypnotic in the evening for several weeks during the attack.

To verify our hypothesis extended studies with family investigations like the ones reported here will be necessary and we therefore recommend such analyses as a routine in all cases of porphyria when circumstances allow having regard to the fact that such investigations require much time and skill. We wish to emphasize however that if properly administered much of the work can be done by secretaries and technicians making the burden on the physicians considerably less than it appears on first consideration.

Further we wish to point out the diagnostic value of family investigations in many cases of paralytic porphyria in which the excretion of metabolites not infrequently becomes normal long before the pareses disappear. We have several examples of this including our case no. 156 (Fig. 1) in which excretion was completely normal when finally studied by adequate methods and the family had several members with slightly increased excretion of metabolites and one with moderately increased. Another example is case no. 163 (Fig. 6) in which only ALA was increased when the urine was subjected to adequate study in the paralytic stage. In this connection it is worth remembering that in South African VP the excretion of PBG and ALA during acute attacks usually becomes normal within a few weeks after the beginning of the attack, normal excretion of PBG and ALA being frequently seen while pareses are still present (5). Thus some of our cases with AIP and low gene penetration are similar to South Africa's VP in this respect.

Family investigations may also be valuable for avoiding an erroneous diagnosis of porphyria. It is now recognized that porphyria may coincide with other diseases which clinically may mimic a porphyric attack—as for example inflammatory spinal lesions (21), polyarteritis nodosa (Becker (1) and Waldenström (17)) and disseminated lupus erythematosus (8) and if such diseases occur

in an unknown latent porphyric constantly excreting high concentrations of metabolites this can easily lead to a false diagnosis of porphyria. But if the porphyric is known beforehand from a former family investigation this error becomes less likely.

Our hypothesis is also supported by the differences between observations on variegate porphyria in South Africa and Sweden as recently expressed by Hamnström *et al.* (7). The South Africans (2, 3, 4, 6) find that increased faecal porphyrin excretion is practically constant in VP carriers while the Swedish investigators could not confirm this and found a carrier in their family II who was normal in all the chemical tests as well as one with normal PBG and increased ALA in their family C.

Danish experience of VP is limited as our material consists of only four VP patients of whom two are adopted children. Three of these cases were studied by the writer and in two faecal porphyrin excretion was followed through several years and found to be intermittent. In one a family investigation was performed. All members of two generations a total of nine subjects were studied and only the patient showed elevated faecal porphyrins. Two of the others showed slightly increased ALA the remainder was normal. These observations suggest definite differences between VP in Denmark and South Africa. Watson (20) too emphasized the differences in VP as described by South African workers and the mixed porphyria cases from his United States material which resembled them. One of the Danish cases of VP (case no. 118) is of special interest (Chief physicians O. Remvig, Fysiurgisk Hospital, Hørsholm and H. Brodthagen, Finsensinstituttet, Skin Department placed this patient at our disposal).

Woman, born 1961 adopted child developed a severe attack of paralytic porphyria in October 1964. She was of dark hue but had no hypertrichoses. During the beginning of the attack her urine PBG was 2.15 and ALA 130 mg/100 ml and her faecal porphyrin 1700 μ g/g dry matter consisting of coproporphyrin without demonstrable dicarboxylic porphyrins. Typical crystalline coproporphyrin III ester was prepared from one week's collections of her faeces in Nov. 1964 and May 1965 c. 50 mg on each occasion. She recovered in the course of one year being discharged from hospital with only partial paresis of the left thumb remaining in Nov. 1965. During the first year of her disease PBG and ALA had been elevated and on discharge PBG was still 2.2 mg per 100 ml. There had been a few episodes of abdominal pain, but no fresh

during the phase of recovery from Nov 1964 to Nov 1965. During a holiday in Spain she developed typical cutaneous porphyria in the summer of 1966. Before that time had every summer exposed herself freely to the sun in Denmark without developing blisters. In April 1967 showed coproporphyrin 370 and protoporphyrin $\mu\text{g/g}$ dry matter and since January 1968 only δ -ALA porphyrins were found in the faeces in concentrations of 300–500 $\mu\text{g/g}$ dry matter on four occasions. In January 1968 her urine was studied on eight occasions and showed negative screening test for porphyrins. 1 normal PBG and ALA.

This case thus shows a definite shift of the porphyrin excretion from coproporphyrin and δ -ALA porphyrins simultaneously with the development of cutaneous porphyria and disappearance of the excretion of porphyrin precursors. It is interesting that Watson (20) often found increased faecal uroporphyrin in his patients with acute porphyria, while this finding is exceptional in Denmark, where we have only found it in faeces on two occasions, as well as in England (3). On the other hand uroporphyrin is not infrequently excreted with the faeces during attacks (VP 14 15).

Further the very high sensitivity of carriers of P to barbiturates (2) seems to be peculiar to the ge South African Boer family and it is reasonable to suggest that this family like the large family of Northern Sweden is characterized by exceptionally high gene penetration, high excretion of metabolites and high drug sensitivity. Finally it has recently been pointed out that the more rare form of porphyria HCP can be a single entity but shows considerable variations from country to country or more correctly family to family (11).

CONCLUSION

We wish to stress the value of performing family studies as often as possible and to include exchange chromatographic determinations of PBG and ALA in these investigations. Without these analyses many carriers will inevitably be lost (27). It is also important always to include these analyses in order to find out to which of the groups of hepatic porphyria (AIP, VP, HCP) the patient belongs. As pointed out above, family investigations have a definite diagnostic value besides their value in preventing attacks in carriers.

Finally family studies help in evaluating the degree of gene penetration in the patient's family and consequently according to our hypothesis enable us to predict the degree of drug sensitivity. The latter might be of interest in epileptic porphyrics, diabetic porphyrics (oral antidiabetics of sulphonamide nature), patients requiring griseofulvin and women requiring oral contraceptives.

A complete porphyrinological investigation thus comprises screening of urine and faeces for porphyrins and quantitative analysis if positive tests are found, as well as ion exchange chromatographic determination of both PBG and ALA. Further it is recommended to include thin layer chromatographic study of the porphyrins by the talc method which is easy, rapid and cheap (26, 28). If the porphyrins are extracted from faeces or urine with a mixture of equal volumes of amyl alcohol, glacial acetic acid and ether and from this extract with 1 M HCl which is then applied to chromatographic talc plates, beautiful chromatograms can be obtained in 10–15 min in which also 7-carboxyl porphyrin, diagnostic of PCT, can be clearly separated from uroporphyrin (29).

ACKNOWLEDGEMENT

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Further the very high sensitivity of carriers of P to barbiturates (2) seems to be peculiar to the South African Boer family and it is reasonable to suggest that this family like the large family of Northern Sweden is characterized by exceptionally high gene penetration, high excretion of metabolites and high drug sensitivity. Finally it has recently been pointed out that so the more rare form of porphyria HCP can be a single entity but shows considerable variation from country to country or more correct from family to family (11).

CONCLUSION

We wish to stress the value of performing family investigations as often as possible and to include ion exchange chromatographic determinations of PBG and ALA in these investigations. Without such analyses many carriers will inevitably be lost (1, 27). It is also important always to include chemical analyses in order to find out to which of the groups of hepatic porphyria (AIP, VP, HCP) the patient belongs. As pointed out above, family investigations have a definite diagnostic value besides their value in preventing attacks in carriers.

Finally family studies help in evaluating the degree of gene penetration in the patient's family and consequently according to our hypothesis enable us to predict the degree of drug sensitivity. The latter might be of interest in epileptic porphyrics, diabetic porphyrics (oral antidiabetics of sulphonamide nature), patients requiring griseofulvin and women requiring oral contraceptives.

A complete porphyrinological investigation thus comprises screening of urine and faeces for porphyrins and quantitative analysis if positive tests are found as well as ion exchange chromatographic determination of both PBG and ALA. Further it is recommended to include thin layer chromatographic study of the porphyrins by the talc method which is easy, rapid and cheap (26, 28). If the porphyrins are extracted from faeces or urine with a mixture of equal volumes of amyl alcohol, glacial acetic acid and ether and from this extract with 1 M HCl which is then applied to chromatographic talc plates, beautiful chromatograms can be obtained in 10–15 min in which also 7-carboxyl porphyrin, diagnostic of PCT, can be clearly separated from uroporphyrin (29).

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INCREASED ENTERIC FORMATION OF HISTAMINE IN CHRONIC PANCREATITIS

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Abstract Patients with chronic pancreatitis in contrast to patients with acute pancreatitis were found to have increased excretion of conjugated histamine in urine (7 of 9 patients). A close correlation existed between the amounts of conjugated histamine in urine and the concentration of histamine like activity in the feces. Fecal samples from patients in contrast to samples from controls, possessed a capacity to form histamine like activity from histidine and *N*-acetyl-L-histidine. Sterile filtrates on the other hand possessed no such ability. Feces from patients had a significantly lower pH than control feces. This may be a predisposing factor for the observed histamine formation since the bacterial L-histidine decarboxylase has a low pH optimum.

It is concluded that the excess conjugated histamine in urine in these patients originates in the lumen of the gastrointestinal tract.

Various histamine metabolites seem to be present in human urine: 1,4-methylhistamine (8), mono-di- and trimethylhistamine (methyl groups in the side chain of histamine) (12), 1,4-methylimidazole acetic acid (1,5-methylimidazole acetic acid (10, 28)), *N*-acetylhistamine (29) and also probably 4-(5-imidazolyl)-ethane-2-ol (18). Injected labelled histamine is also converted to imidazole acetic acid and imidazole acetic acid riboside and excreted as such in the urine (20).

N-acetylhistamine is apparently a rather unimportant urinary metabolite of parenterally administered labelled histamine (*N*-acetylhistamine/histamine ratio in urine $1 - < 0.2$ (13, 20)). The so-called conjugated histamine in the urine which signifies the increment in biologically active histamine upon acid hydrolysis appears spontaneously in the urine in a relatively higher proportion (mean conjugated histamine/free histamine ratio in healthy individuals 2.4 (23)). Nevertheless the urinary conjugated histamine seems to consist

wholly (or partly?) of *N*-acetylhistamine. The explanation of this apparent inconsistency may be that the conjugated histamine in urine (and *N*-acetylhistamine) has a dual origin both endogenous and exogenous (6).

There seem to be at least three different types of increased urinary excretion of conjugated histamine in man:

- 1 Increased excretion secondary to ingestion of histamine (1)
- 2 Increased excretion secondary to ingestion of *N*-acetylhistamine (25)
- 3 Increased excretion of conjugated histamine in the urine as encountered in some patients with myotonic dystrophy (26)

In myotonic dystrophy there is a close correlation between the urinary excretion of conjugated histamine and the fecal excretion of histamine like activity (26). The primary abnormality in this chain of events appears to be increased formation of histamine like activity in the intestinal lumen. The formed histamine is conjugated and absorbed or vice versa and then excreted in the urine in the conjugated form. These findings add weight to the view (4) that there is an intestinal dysfunction as well in myotonic dystrophy.

It may be anticipated that some disorders primarily affecting the gastrointestinal tract could have the same abnormality of histamine metabolism. Therefore in order to obtain information regarding the specificity of increased urinary excretion of conjugated histamine in myotonic dystrophy a study of the urinary excretion of conjugated histamine in gastrointestinal disorders was carried out (27).

Table I *Urinary and fecal excretion of histamine in patients with chronic pancreatitis*

Case no	No of obs urine	Urinary hist $\mu\text{g}/24 \text{ h}$				HA ^a Mean (range)	Fecal pH		Commentary
		Free		Conjugated			Mean (range)	Mean (range)	
		Mean	Range	Mean	Range				
1	8	40	23-90	2850	920-7100	88 (47-140)	6.2 (6.0-6.6)		
2	2	16	8-23	1250	600-1900	—	—		
3	5	75	11-160	510	340-1000	24 (13-38)	6.2		
4	4	3	<2.4-6	400	87-350	3.7 (0.5-6.9)	6.3 (5.6-7.0)	Gastric resection 17 y previously	
5	1	34	34	200	200	—	—		
6	3	20	<6-30	140	38-220	0.4	6.1		
7	2	27	6-47	100	55-150	—	—		
8	2	8	4-11	36	25-47	0.0	5.4	Pulmonary symptoms Sweat test normal	
9	3	14	12-17	410	330-480	0.14 (0.03-0.23)	7.0 (6.9-7.2)	No steatorrhea at time of study Gastric ulcer Laparotomy Cystic pancreas Chr inflammation	
10	1	4	4	74	74	—	—	No steatorrhea at time of study Diagnosis only probable	
11	2	14	5-22	155	140-170	1.8	6.9 (6.8-6.9)	Diagnosis only probable	
12	1	7	7	43	43	—	—	Diagnosis only probable	
Control series (23)		12.6	2-31	30.0	1-99	<0.17			

^a Histamine like activity in the feces in $\mu\text{g base/g wet weight}$

Contrary to expectation normal or only sporadically elevated excretion was found in all disorders examined except one in patients with steatorrhea due to chronic pancreatitis (a total of 4 patients) there was significantly increased excretion of conjugated histamine.

The present communication summarizes studies intended to substantiate this finding and to elucidate the mechanism producing the increased output of conjugated histamine in urine in chronic pancreatitis.

MATERIAL

Eight cases considered to suffer from chronic pancreatitis with steatorrhea were studied. Patients nos 9 and 10 (Table I) did not have steatorrhea at the time of study. Due to the paucity of cases three cases with only probable chronic pancreatitis were included (viz. nos 10-12).

As is evident from Table VII the patients with acute pancreatitis were heavily medicated and this might have influenced the results of the histamine studies. In particular antihistaminics, cortisone and meperidine are pertinent in this connection. Antihistaminics influence the biological assay of histamine; cortisone increases the excretion of histamine in the urine (17) and meperidine is a releaser of histamine (31).

Some of the patients with acute pancreatitis were in poor condition particularly no. 16 but all survived the attack. Patient no. 15 died from mesenteric vein thrombosis approximately three months later.

No restrictions were given as to diet during study. Most patients with chronic pancreatitis ingested a low fat diet. Most patients with chronic pancreatitis also used Combizym®.

METHODS

Free and conjugated histamine in urine

With some minor modifications Dunér and Pernow's method (5) has been employed. Urine was collected in 24 hour portions, but the collections were not carried out in a metabolic ward and urinary creatinine determinations were not made. To check the completeness of collection culture and macroscopical examination of urine were carried out in some patients and there were no signs of infection.

Free and conjugated histamine in gastric and duodenal juice

These parameters were measured as described elsewhere (26) using samples obtained during secretin tests prior to injection of secretin.

Histamine like activity in the feces

For the estimation of the free fraction a method described in detail elsewhere (27) has been employed. This

method is inaccurate for estimating the conjugated fraction in the presence of high levels of histamine like activity. In order to identify or quantify the conjugated fraction in stool samples three methods were used.

Paper chromatography (see later) for qualitative purposes.

The free and conjugated fractions were separated on an Amberlite IRC-50 column as described elsewhere (method 2/26) and subsequently estimated semiquantitatively.

The histamine like activity in the fecal sample concerned was estimated. Another aliquot of the homogenized fecal sample was incubated with diamine oxidase in order to inactivate the histamine like activity partially or completely (this was checked by a biological technique) after which the regular technique (22) for quantifying conjugated histamine was adopted.

Fecal incubations

Incubations were carried out in the following manner. The whole fresh specimen was homogenized and an aliquot approximately 5 g was mixed carefully with 15 ml distilled water. Incubations were carried out aerobically without stirring at 37°C for 20–24 h with 1) 300 mg of L-histidine monohydrochloride monohydrate or 2) 300 mg of N-acetyl L-histidine 3) C-histamine dihydrochloride 5–100 µg (specific activity 116 µCi/mg) or 4) L-histidine 2–60 mCi (specific activity 35 mCi/mmol). Biological assay and autoradiography showed that the batches of both conventional and labelled L-histidine (30) and N-acetyl L-histidine contained only negligible quantities of histamine. In other experiments 1–3 g of patients' feces and 1–15 g of control feces were mixed, homogenized and incubated in the same way.

The pH in the incubation mixtures was not adjusted after addition of the chemical substances.

After incubation the mixture was centrifuged and the supernatant fluid filtered by suction in a Buchner funnel. The pH was adjusted to pH 7.4 before direct assay on the guinea pig ileum. A few samples were subjected to the aforementioned extraction procedure (72) used for histamine like activity in the feces.

All values for histamine refer to the base and almost invariably represent the mean of duplicate analyses. Although recovery studies have been carried out systematically no corrections have been made for losses during the procedures.

Fecal pH in freshly expelled whole sample homogenates (weight of feces in g × 3 = ml of distilled water) was measured potentiometrically with a glass electrode (Radiometer Copenhagen).

Indican excretion in urine was measured as outlined by Meiklejohn and Cohen (13). Urine was collected with 60 ml 12 N hydrochloric acid instead of toluene as recommended by the authors.

Paper chromatography

The following systems were used for two-dimensional descending paper chromatography of fecal extracts (Whitman's paper no. 1): system 1) n-butanol/acetic acid/distilled

water (4:1:1 v/v) and system 2) n-butanol saturated with 20% NH₄OH. As histamine and 14-methylhistamine are poorly separated in this chromatographic system, another moving phase was used to separate them: 3) phenol/chloroform/ethanol/ammoma (14:14:10:2 v/v (T. White personal communication)). It was not necessary to carry out desalting of the fecal extracts since the moving rates of internal standards were not notably altered.

Fecal extracts were prepared as described under fecal incubations. Generally 50 µl of the filtrates were spotted on the chromatograms. The chromatograms were stained with either diazotized p-toluidine or diazotized sulphadiazide.

Kodak X-ray films were exposed to the radioactive chromatograms for 4–6 weeks. No quantification of the radioactive metabolites was attempted in these experiments.

L-histidine loading orally

After a control day 10 g L-histidine monohydrochloride monohydrate was ingested with 200 ml tap water at 8 a.m. after overnight fasting. On two occasions the oral dose was ingested also on the subsequent morning. The patients ate their regular meals during the study.

Oral sulfa treatment

Phthalylsulphathiazole (AFI-Fthalyl 3) was administered with the following doses: 7 g initially then 10 g per day for 5 days. No stool cultures were taken to check the effect of the treatment.

RESULTS

Patients with Chronic Pancreatitis

Histamine in urine

The findings are condensed in Table I. Seven of eight patients whose diagnosis was considered rather definite had an augmented output of conjugated histamine (88%).

One of three patients with only probable diagnosis also had this anomaly. In none of the patients was the mean excretion lower than the mean control excretion. The difference between patients and controls was statistically significant both for the first eight cases and for the whole series ($P=0.05$).

The free fraction was elevated in three cases with calculations based on unweighted values and in four cases when based on weighted values. All cases with augmented output of free histamine also showed augmented output of conjugated histamine. The mean excretion of free histamine did not differ significantly from the normal one ($P>0.1$). Not only was the excretion of conjugated histamine more frequently elevated than that of free histamine but also quantitatively more

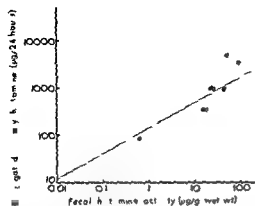


Fig. 1 Logarithmic values for conjugated histamine in urine plotted against logarithmic values for histamine like activity in the feces. Altogether 17 corresponding samples of feces and urine from 7 patients with chronic pancreatitis. Correlation coefficient $r = +0.88$ ($P < 0.001$). Equation of regression $\log \text{conj. histamine urine} = 0.55 \log \text{histamine feces} + 2.15$.

heavily affected. The conjugated/free histamine ratio for the whole present series was 22.8 against a control value of 2.4 (23).

The statistical calculation both for free and conjugated histamine were based on unweighted values. These values were more representative of the material since more samples were obtained from patients with high than from patients with low urinary excretion of the respective substances.

Histamine like activity in the feces

Seventeen samples were obtained from seven patients. With one exception increased values were

Table II Simultaneous estimation of histamine like activity and conjugated histamine in feces from patients with chronic pancreatitis

Case no.	Method no. ^a	Histamine in feces ($\mu\text{g/g}$ wet weight)	
		Histamine	Conjugated histamine
1	2	20	0.5
1	2	91	5.8
1	2	117	14
3	2	9.2	7.8
3	2	8.3	32
3	3	27	8.3
3	3	0.43	0.14

^a See Methods (histamine like activity in the feces).

Table III Histamine in gastric and duodenal juice in patients with pancreatic disorders

Case no.	Diagnosis	Histamine (μg base/ml)	
		Gastric	Duodenal
1	Chronic pancreatitis	$<0.07 / <0.06^a$	$<0.07 / <0.4$
13	Chronic pancreatitis	— / 0.2	—
14	Insulinoma	—	— / 0.0
Control values		$<0.03 / <0.018$	$<0.6 / <0.1$

^a Free histamine/conjugated histamine.

found in every instance of increased excretion of conjugated histamine in the urine. Elevated histamine like activity was present in the corresponding fecal sample (Table II).

The figures for urinary conjugated histamine and fecal histamine like activity in corresponding samples were recalculated to a logarithmic scale and plotted against each other. The correlation between the urinary and fecal parameters was close (Fig. 1).

The contractions of the guinea pig ileum caused by the bioactive substance in feces mimicked those caused by authentic histamine diphosphate in every respect. An antihistaminic agent, diphenhydramine hydrochloride, was occasionally added to the bath during assay. The fact that it antagonized the contractions caused by the eluate and those caused by authentic histamine in the same degree (19) was taken to indicate that the substance tested was biologically identical with histamine. Addition of atropine or dihydroergotamine to the bath was without any influence. Nevertheless, the biological activity was designated histamine like activity since it was more stable on incubation than added histamine and since clearcut results as to the identity of the substance were not obtained by chromatography (see later).

With special methods for separation of histamine and conjugated histamine (see Methods) it was found that a conjugated fraction was present in the feces as well (Table II).

Histamine in gastric and duodenal juice

Normal or only slightly elevated values were found (Table III), the values being of the same order of magnitude as in myotonic dystrophy (26).

ecal incubations

shown in Table IV huge quantities of histamine were formed when feces from patients were bated with histidine. In these experiments the HA was lowered because of the addition of the amino acid. In control experiments however with pH adjusted to 5.5-7.0 an increased formation of histamine also took place. Table IV demonstrates that patients' feces in contrast to control feces could form histamine like activity from *N*-acetyl-L-histidine. In a few experiments sulfa and histidine were added to patients' feces. This led to a low histamine formation even at an pH 7.0. Sterile filtrates (Millipore Filter pore diameter 0.22 μ) of feces possessed no ability to form histamine. Patients' feces seemed to be able to dispose of *N*-acetylhistamine and in the course of this process a free fraction evolved as in control feces.

Inherent fecal histamine like activity proved remarkably stable on incubation under the regular experimental conditions even when the incubation period was prolonged to five days. Inherent histamine like activity on incubation was reduced at a much slower rate than authentic histamine by diamine oxidase.

From Table V it appears that with one exception addition of control feces to feces with high inherent histamine like activity led to a marked reduction of the activity.

Table IV Formation of histamine like activity (HA) in the feces (5 g samples) (chronic pancreatitis) after addition of L-histidine (222 mg of the free amino acid) or *N*-acetyl-L-histidine (237 mg of the free amino acid)

Case no	Control HA	Additional activity after addition of	
		L-histidine	<i>N</i> -acetyl-L-histidine
1	47	26 000	600
1	—	31 000	1460
1	86	31 000	—
1	—	49 000	—
1	—	140 000	—
1	—	40 000	—
1	55	106 000 ^b	1420
3	13	70 000	—
3	22	138 000	—
Control incubations (4) < 5.0		< 0.4	

^a Histamine like activity in μ g base/g wet weight

^b No methylhistamine could be detected in this sample

Table V Influence of control feces on inherent HA. Incubation for 20-24 h at inherent pH

Case no	Feces wt in g		HA ^a	
	Control	Pat	Before incubation	After incubation
1	1	1	30	20
1	3	1	30	17
1	5	1	120	850
1	14	3	28	11
1	5	1	18	85
1	5	2	35	10
3	5	1	22	20
3	15	1	22	< 0.6

^a Histamine like activity in μ g base/g wet weight

Data between solid horizontal lines represent the same fecal specimen from patient as well as from control

Paper chromatography and autoradiography

Extracts from fecal samples with high inherent histamine like activity were submitted to chromatography (systems 1 and 2) and this disclosed that a double spot was present almost invariably. The moving rate of the one spot corresponded approximately to that of authentic histamine di-phosphate (which occasionally also gives a double spot in system 1 (24)) with the following *r*_f values: system 1 approximately 0.08 (and 0.24) and system 2 approximately 0.40. Both spots could be localized by their UV fluorescence and were eluted without staining. Both spots proved to contain biological activity which could not be differentiated from that of authentic histamine by parameters such as addition of antihistamine, atropine or dihydroergotamine to the biological bath during assay.

A spot that on the basis of moving rates could not be differentiated from *N*-acetylhistamine was most often present in patients' feces.

Both conventional and labelled histamine added to patients' feces were degraded but to a markedly less extent than by control feces. A fraction with the same moving rate as *N*-acetylhistamine evolved and so did an unidentified fraction with the approximate *r*_f 1) 0.58 and 2) 0.78. L-histidine, conventional and labelled, was converted to histamine (double spot) and to a much lesser extent to urocanic acid, dihydrourocanic acid and to the aforementioned unidentified spot. Little unchanged L-histidine was left. In control feces the main

Table VI *Urinary and fecal excretion of histamine after oral L histidine loading (chronic pancreatitis)*

Case no		Control day	Loading		Post loading	
			1st day	2nd day	1st day	2nd day
1	Free	90	58	—		
	Conj	7100	4000			
	HA ^a	125	18			
1	Free	29	96	—	19	42
	Conj	1100	3400		1500	1100
	HA	III	23 76 ^b		13	37
3	Free	38	34	34		
	Conj	550	230	150		
	HA	13	—	19		
3	Free	100	93	240		
	Conj	1000	430	760		
	HA	24	65	100		

^a Fecal histamine like activity in μg base/g wet weight^b Two samples on same day

metabolites were urocanic acid and dihydrourocanic acid. The histamine spot was lacking or small and much of the L histidine remained unchanged after incubation (24).

No methylhistamine was detected in a fecal sample with high amount of generated histamine like activity.

Oral sulfa treatment

During sulfa treatment in patient no 1 the fecal histamine like activity dropped to 17—the lowest observed control value in this patient being 47 $\mu\text{g/g}$ —whereas the conjugated fraction in urine dropped to 340 the lowest control value being 820 $\mu\text{g}/24$ h. Patient no 3 developed frequent diarrhea during sulfa treatment which therefore had to be discontinued. In this patient only a slight decrease occurred in the fecal and urinary parameters.

Oral L histidine loading

The two studies in patient no 1 showed opposite results both with regard to urinary free and conjugated histamine and with regard to fecal histamine (Table I). Patient no 3 developed frequent diarrhea during both studies the diarrhea starting less than 1 h after administration of L histidine, so probably most of the amino acid was lost via the fecal route.

Fecal pH

Fecal pH was measured in samples from altogether seven patients. In five cases whose diagnosis was considered rather definite the mean pH was 6.0 (Table II) against a mean control value (26) of 7.13 ($P < 0.01$). In the whole series the mean pH was 6.3. It is noteworthy that in patient no 4 a fecal pH of 7.0 was accompanied by a conjugated histamine excretion of 87 $\mu\text{g}/24$ h whereas the fecal histamine like activity was 0.6 $\mu\text{g/g}$. The corresponding figures accompanying a fecal pH of 5.6 were 240 $\mu\text{g}/24$ h and 6.9 $\mu\text{g/g}$.

Urinary excretion of indican

Three samples from patients nos 1 and 3 were studied and a mean excretion of 65 mg/24 h was found (range 88–53 mg/24 h). In a control series (26) the values ranged from 41–113 with a mean of 62.9 mg/24 h.

Urinary Output of Histamine in Patients with Various Other Disorders of the Pancreas

Acute pancreatitis

The results are presented in Table VII. The pattern was entirely different from that of chronic pancreatitis in that none of the patients displayed increased urinary excretion of conjugated histamine. The excretion of free histamine was increased in two patients and markedly so in patient no 15 in spite of medication with an antihistaminic drug. Worthy of comment is also the increased fecal histamine like activity in this patient. In our experience increased fecal histamine like activity is always accompanied by increased urinary conjugated histamine levels. We have seen only one exception to this previously (27) and both in the previous and the present case it may be due to methodological errors: the method used is not very accurate for estimating relatively low quantities of conjugated histamine in the presence of high quantities of free histamine since the conjugated fraction is calculated by difference (total histamine – free histamine = conjugated histamine) and the recoveries of both N acetyl histamine and histamine diphosphate vary considerably (23).

Cancer of the pancreas and mucoviscidosis

Normal excretion was found in the two patients with cancer of the pancreas whereas the two

Table VII Urinary excretion of histamine in patients with acute pancreatitis

Case no	Sex	Histamine in urine ($\mu\text{g base}/24\text{ h}$)		Drugs ^a	Urinary diastase
		Free	Conjugated		
15	♀	250	32 (12 ^b)	Chlorpromazin digitalis, penicillin streptomycin phenergan ⁸	256
16	♀	<20	14	Trasyol ⁸ chlorpromazin insulin penicillin cortison, meperidine	2048
17	♀	8	39	—	Three days after normalization
18	♂	49	34	—	10-4 on day before collection. 8 on collection day
19	♂	9	7	—	512 two days prior to collection 32 on day after collection
20 ^c	♀	3	—	Tetracycline	4096
21	♀	7	18	—	Five days after normalization
—	♀	Trace	22	Penicillin, streptomycin	512

^a Drugs given during or just before the collection of urine

^b Histamine-like activity in feces $\mu\text{g base/g wet weight}$.

^c This patient also had cholecystitis.

children with mucoviscidosis showed moderately increased excretion of conjugated histamine (Table VIII)

DISCUSSION

The present study confirms the previous observation (27) that in patients with chronic pancreatitis increased urinary excretion of conjugated histamine frequently coexists with increased fecal output of histamine like activity. In cases in which the diagnosis was considered rather definite the incidence of augmented excretion was high.

As far as the free fraction in urine is concerned increased excretion was observed in some cases with increased excretion of conjugated histamine.

The observed features may serve in the differential diagnosis of steatorrhea. In idiopathic

SHIAA excretion is elevated (11-14)

) whereas conjugated histamine excretion in urine mostly is normal. In chronic pancreatitis

HIAA excretion in urine is normal (11-14) but conjugated histamine excretion in urine is

evated and so is the histamine like activity in feces. It should be stressed that as for histamine there will be exceptions. With Arjona et al's

method (3-22) the estimation of histamine like activity in the feces is a rather simple procedure.

The close correlation between the excretion of conjugated histamine in urine and histamine like

activity in feces points to a cause and effect relationship between the two phenomena. The following reasoning may have a bearing on what is the primary abnormality in this sequence of events. When histamine is ingested (1) or administered into the gastrointestinal tract (25) the following pattern is found: the fecal output of histamine rises and so does the urinary output of conjugated histamine whereas the output of free histamine does not increase significantly (the conjugated/free histamine ratio increases markedly). The similarity of this pattern with that observed in chronic pancreatitis indicates an intestinal origin of the histamine in this disorder.

Table VIII Urinary excretion of histamine in patients with cancer of the pancreas and mucoviscidosis

Case no	Sex	Diagnosis	Urinary histamine ($\mu\text{g base}/24\text{ h}$)	
			Free	Conjugated
23	♂	Cancer of the pancreas	2	5
24	♂	Cancer of the pancreas	5	24
25 A	♂	Mucoviscidosis	5	5
25 B	♀	Mucoviscidosis	5	11
6	♂	Mucoviscidosis	9	36
Control values in 8 children <9 y mean and range (27)			5.3 (2-11)	5.1 (0-10)

The children aged 4-5 (no. 25) and 7 (no. 6)

The following possibilities merit consideration to explain the presence of increased quantities of histamine (or *N*-acetylhistamine) intraluminally in the intestine in this disorder: 1 Ingestion of histamine 2 Release of histamine into the intestinal lumen 3 Production of histamine intraluminally 4 Decreased breakdown of intraluminally formed histamine. Furthermore, ingestion of *N*-acetylhistamine must be considered.

There is little to support the idea that histamine or *N*-acetylhistamine preformed in the food (2) might be the underlying cause since most of the patients were given regular ward diets during study. Further, patient no. 1 was studied for three successive days with a regular mixed diet without any change in the fecal or urinary excretion of histamine. There is further evidence to refute the possibility of *N*-acetylhistamine ingestion. Ingestion of *N*-acetylhistamine does not lead to increased fecal output of histamine-like activity or conjugated histamine, whereas our patients showed increased fecal excretion of these substances.

Moreover, the increased faculty of patients' feces to form histamine (in *in vitro* studies) cannot be explained by an increased flow of histamine to the lumen of the gut, be it by release of mucosal cells or by ingestion. In that case these patients must have *in vivo* abnormalities of histamine metabolism in the gut, and that seems highly unlikely. If decreased catabolism of histamine were to explain the inherent histamine-like activity, it is inferred that formation of histamine normally takes place in the lumen of the gut, but that the formed histamine is inactivated before it can be detected. This possibility seems unlikely since there is evidence that intraluminal formation of histamine in man is limited (24). The fact that ^{14}C -histamine was catabolized rather efficiently by patients' feces in *in vitro* studies tells against decreased catabolism as the only cause of the abnormally high histamine-like activity in patients' feces. (For further discussion regarding the stability of inherent histamine in feces, the reader is referred to (24).)

By exclusion, it seems logical to conclude that increased intraluminal formation of histamine (i.e., histidine decarboxylation) seems to be the cause of the urinary abnormality in these patients. Many intestinal bacterial species possess a potent histidine decarboxylase. The impetus of oral sulfa

in patient no. 1 may be due to reduction of such bacteria in the intestinal lumen. The fact that only a reduction and not a normalization of urinary and fecal histamine was achieved with sulfa might possibly be ascribed to too low a dosage of sulfa since the quantities of feces in this patient amounted to 1 kg or more per day. The incubation studies with sterile filtrates of feces also pointed to an important role for intestinal bacteria in this connection. The most plausible explanation of the ability of control feces to reduce inherent histamine-like activity in patients' feces is that the effect is brought about by the bacterial flora as such.

The bacterial decarboxylase has a low optimal pH 2.5–3.0 (9). In cases of steatorrhea due to chronic pancreatitis, the fecal pH was decreased and this might facilitate histamine formation. The pathogenesis for the increased intraluminal histamine formation may therefore be the following: undigested protein (in increased quantities, since steatorrhea is present?) reaches the large bowel where the pH is low (lack of bicarbonate secretion by the pancreas?). Certain bacterial strains with the actual decarboxylase in their potential enzymic constitution will proliferate due to the preponderance of the substrate (16). L-histidine is decarboxylated and histamine is formed. The change in bacteria is probably secondary to the change in the intraluminal milieu.

The metabolic aberration may thus differ somewhat from that in myotonic dystrophy, in which the fecal pH was normal and the exocrine pancreas function seemed to be normal.

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TWO METHODS FOR PRODUCING IN ANIMALS A MACROGLOBULIN SIMILAR TO THE RHEUMATOID FACTOR

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Abstract Two methods for provoking a haema glutinatin macroglobulin identical or similar to the Rheumatoid factor (RF) are described. The macroglobulins were obtained by injections of *Diplostreptococcus agalactiae* animals mainly white rats (b) by injecting into rats antibody to RF (anti RF) or the active fraction of antibody. The anti RF was produced by immunizing rabbits against RF. Both (a) and (b) have given rise to circulating macroglobulins showing high titres (up to 10^4) in the sheep cell test. The titre is much more stable when using method (a). Using method (b) the show sudden variations with sometimes total disappearance of the haemagglutinating macroglobulin.

The anti RF serum produced neutralizes the RF component in precipitation tests. The active principle of anti RF is a fraction showing a sedimentation constant of about 7 S, i.e. exactly the same as that of the common γ G. Comparing the 7 S which has the property of neutralizing RF completely in unheated state and the common γ G (IgG) by immunoelectrophoresis, the first does not give rise to any precipitation line with anti- γ G while of course the second provokes a typical line. The RF neutralizing globulin seems to be a hitherto unknown 7 S globulin. The same is true of the isolated haemagglutinating fragment of RF. Details of the latter type of 7 S globulin were given in earlier papers.

Thus, the active fractions both of RF and anti RF have been isolated and studied. They constitute two members of the large 7 S family which certainly contains an enormous number of 7 S gamma globulins (γ G or IgG) with different properties. As was pointed out earlier (10) there also exist a great number of different macroglobulins.

Some years Svartz has been engaged on trials in the experimental production of chronic polyarthritis (5, 15). Later these investigations were continued with experiments aimed at provoking in animals a haemagglutinating macroglobulin (7, 8, 9) identical or similar to the Rheumatoid factor (RF).

For this purpose injections of different types of

bacteria were tried. The most favourable results were obtained by using a strain of streptococcus B known earlier as *Streptococcus agalactiae* which is one of the main causes of mastitis in cattle. The *Streptococcus agalactiae* is here called *Diplostreptococcus agalactiae* since even in fluid medium it has a pronounced tendency to appear in diploform (Fig. 1).

It was stated that these cocci could fairly often be cultivated from the human throat and more often from RA patients than from healthy individuals. In the course of the years many different methods of cultivating these bacteria were tried. As regards some strains the addition of DNA seemed to promote the growth. Our present methods of isolating *agalactiae* and of producing haemagglutinating macroglobulins will be described below.

As was reported in this journal (14) a 7 S gamma globulin γ G or IgG was isolated from anti Rheumatoid factor serum (anti RF serum) produced in rabbits. This IgG showed the capacity in non-aggregated state of completely neutralizing the Rheumatoid factor. The type of IgG in question was called IgR in order to emphasize its connection with the RF and Rheumatoid arthritis (RA). The present paper will describe attempts to produce a macroglobulin of type RF by injections of anti RF into animals.

MATERIAL AND METHODS

For the production of a haemagglutinating macroglobulin by means of bacteria the following method has been used during the last two years.

Diplostreptococcus agalactiae (belonging to group B streptococci (Fig. 1)) obtained from the throat of patients with Rheumatoid arthritis, was first cultivated on 1.0 ga

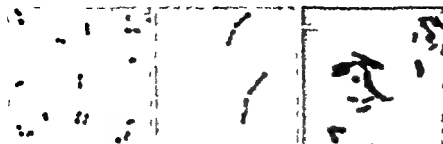


Fig 1 Microphotos of different shapes of *Diplostreptococcus agalactiae* (DSA) a coccus which is extremely pleomorphic

lactose broth or on blood agar or serum agar containing 1% galactose. After 24–48 h at 34°C subcultures were made on blood agar plates with 1% galactose. The cocci were obtained in a pure state by spreading on blood plates in series.

A platinum loop from a 24–48 h blood agar culture was emulsified in 2 ml saline. From this suspension 0.2–

0.3 ml were injected intraperitoneally (sometimes intravenously) into white rats of type Sprague-Dawley simultaneously with the first or second intraperitoneal injection. 0.2 ml of the same suspension was injected into or in the neighbourhood of the epiphyseal plate of a knee-joint. This type of injection was repeated 1–3 times during the injection period which usually lasted for 6–8 weeks. For details see Table I. If the haemagglutination titre was not satisfactory after 8 weeks the injections were continued. The titre of haemagglutination was checked by the sheep cell test before and three or more times during the course of the injections. Pigs and rabbits were treated in the same way but with higher amounts of bacterial suspension according to body weight.

The other method for producing RF-like macroglobulins was quite different. The Rheumatoid factor (RF) was isolated from Rheumatoid arthritis serum by a method published in JAMA 1965 (10) and elsewhere. The isolated RF was injected into rabbits twice at an interval of about three weeks using the common method for producing antibodies. An antibody with high titre and completely neutralizing the RF was usually found after three–four weeks. The anti-RF serum did not contain any RF at least not at the time when the rabbits were killed by bleeding. The serum obtained was used for several purposes. In this paper one type of experiments will be described. The anti-RF serum was injected into rats and rabbits (see Results). The injections were repeated twice or more. The optimal number of injections has not yet been found but it seems necessary to give at least three injections otherwise the typical macroglobulin disappears rapidly.

Sera from the injected rats and rabbits were investigated for the presence of RF-like macroglobulins by sheep cell tests and immunoelectrophoresis. Experience showed however that rabbits could not be used for this purpose.

The next step consisted of trials in isolating the fraction of the anti-RF serum which gives rise to neutralization of RF (see the aforementioned article (14) in Acta med scand). As reported earlier the RF neutralizing factor was mostly found in the supernatant from the cold precipitate of anti-RF serum and was purified by chromatography. The RF neutralizing fraction of anti-RF was injected into rats in the same way as the original anti-RF serum.

Table I Example of the development of a haemagglutinating macroglobulin in rat (Sprague-Dawley) after injections of DSA

	Haemagglut titre ^a (sheep cell test)	Injections (strain E 7)
1966		
June 1	0	Intraperit
June 2		Intraperit and epiph plate
June 7		Intraperit
June 9		Intraperit and epiph plate
June 11 14 17		Intraperit
June 20		
June 21	0	Intraperit and epiph plate
July 5	1/16	Intraperit
Aug. 30	1/64	
Oct. 18		Intraperit
Nov. 11	1/256	
Nov. 30	1/512	
1967		
Jan. 9	1/512	
March 14	1/256 (haemolys)	
April 4	1/512	Epiphys plate
April 20		
Sept. 11	1/512	
1968		
Febr. 2	1/256 (haemolys)	
March 11	1/128 (bad condit treatment with vitamins)	
May 31		Died

^a Considered negative up to a titre of 1/32.



Fig 2 X ray pictures of the articular knee joints of a rabbit which was given injections of DSA
Magn 1 \times

RESULTS

As already mentioned it has proved possible to produce a haemagglutinating macroglobulin in animals particularly white rats by injections of *Diplostreptococcus agalactiae* (DSA). The haemagglutinating macroglobulin has the same properties as the Rheumatoid factor e.g. as regards the sheep cell test (Waler Rose), sedimentation constant on ultracentrifugation and immunoelectrophoresis.

The noteworthy circumstance was discovered that while a marked arthritis often progressive during a year or more appeared in rabbits (Figs 2-3) it was found almost impossible to provoke a haemagglutinating macroglobulin in this type of animal. The highest titre obtained with Waler Rose's test in experimental rabbits did not amount to more than 1:64 i.e. only a slightly positive reaction. As regards rats the reverse conditions were found: the arthritis was often slight but the titre of haemagglutinating macroglobulin high e.g. up to 1:1024. In some rats however signs of pronounced arthritis occurred (Fig. 4). Whether the haemagglutinating factor produced in rats is identical or whether small dissimilarities exist in comparison with the RF has not yet been estab-

lished with certainty. For this purpose the molecular structure has to be studied.

Some pictures may illustrate the production of RF by injection of DSA. Table I has already been referred to on page 136. Table II shows the course of bacterial injections and the results of the sheep cell test in one of 120 rats. This table also shows that it was necessary to give extra injections when the haemagglutination titre showed a tendency to drop. Table III demonstrates an experiment in



Fig 3 Same rabbit as in Fig. 2 X ray of one of the shoulder joints. Magn 1 \times



Fig 4 Arthritis in a white rat injected with DSA

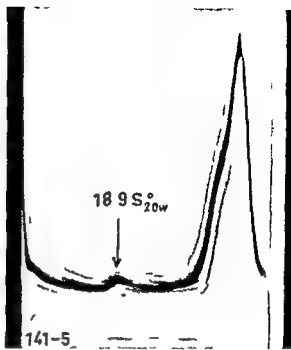


Fig 5 Ultracentrifugation of serum from the rat in Table III Nov 9 1960 The haemagglutinating macroglobulin had an S-value of 18.9

which cortisone injections were given at the same time as intraperitoneal injections of the cocci. As would be expected no influence on the production of RF was found. Fig 5 shows ultracentrifugation of the serum of this rat and Fig 6 granulation tissue in the neighbourhood of the joint capsule. These changes have here the character of vasculitis and perivascularitis.

An X ray picture of an affected joint of a rat has already been shown in Fig 4.

As reported above the experiments from earlier years were performed mainly on rabbits and often gave rise to an advanced and progressive arthritis (Fig 2). Fig 7 shows granulations in the joint capsule from a rabbit which had received injections of *Diplostreptococcus agalactiae*. During a period Svartz had in her animal department several rabbits which had had chronic progressive arthritis during one year or more. Later when the interest was focused rather on the possibility of producing a haemagglutinating macroglobulin by means of bacteria investigations were made also on other animals. We already knew at that time that guinea pigs were out of the question since their sera mostly give rise to a positive Waaler Rose's test. Investigations were made on white rats, pigs, dogs

Table II Production of haemagglutinating macroglobulin in rat by another strain of DSA than in Table I

	Haemagglut titre (Sheep cell test)	Injections (strain Ek)
1966		
June 1	0	Intraperit
June 2		Epiphys plate
June 7		Intraperit
June 9		Intraperit and epiph plate
June 11 14 17		Intraperit
June 21	1 32	Intraperit and epiph plate
July 5		Intraperit
Aug 30	1 128	Intraperit
Sept '71	1 32	Intraperit
Oct 4	1 64-1 1.8	
Nov 21	1 32 (haemolys)	
Nov 30		Intraperit
1967		
Jan 17	1 1074	
March 14	1 256 (haemolys)	
April 4	1 256 (haemolys)	
April 20		Epiphys plate
Sept 11	1 512	
Sept 14		Killed (5 ml serum obtained)



Fig 6 Granulation tissue in the neighbourhood of a joint capsule of the rat in Table III

sheep etc. It was soon found that rats gave the best results.

Up to 1964 about 30% of the rats showed a positive sheep cell test after injections of agalactiae. During the last three-four years the percentage has after methodological modifications amounted to between 50-60. The result of the sheep cell test has as in human beings been considered negative up to a titre of 1:32.

A few comments should be made concerning the different types of injections which have been tried: intraperitoneal, intravenous, subcutaneous injections and injections into the joint and in or in the neighbourhood of the epiphyseal plate of a kneejoint. The reason why this latter method was tried was that we had found by autoradiographic investigations performed in collaboration with the Veterinary School that the Rheumatoid factor has a special affinity for the epiphyseal plate (10, 12). It is too early definitely to judge the value of this method but we have the feeling that it has improved our results. The intravenous injections

Table III An experiment with intraperitoneal injections of DSA (Wall) in rat combined with cortisone injections during the first period

	Haemagglut titre (Sheep cell test)	Injections (strain Wall)
1959		
Nov 25	Neg.	40 intraperiton injections
1960		were given. During the first
Jan 4	1:32	period cortisone inj. were
Jan 7	1:32	given simultaneously
Febr 6	1:32	
March 3	1:64	
March 21	1:512	
April 5	1:256	
Apr 125	1:756	
May 16	1:128	
June 7	1:64	
June 0	1:18	
Aug 8	1:18	
Sept 13	1:756	
Nov 9	1:256	
Nov 26		Died

are not superior to the intraperitoneal. Our earlier and present experiences have shown that subcutaneous and intramuscular injections are less effective.

As has been reported in JAMA and elsewhere (10, 11, 13) the entire RF is not needed for the production of a positive sheep cell test (Svartz) but only a small fragment of it with a sedimentation constant of about 7S (Fig 8). In spite of the fact that the haemagglutinating fragment has the same sedimentation constant as the common 7S or γ G, it has quite different properties otherwise. A description of the haemagglutinating fragment (subunit) of the RF has appeared in Bull Acad nat Med (Paris) 1967 (11) and elsewhere.

It was of course important to know whether the experimentally produced haemagglutinating macroglobulin in rats contained a similar haemagglutinating 7S-fragment to that of the human RF. Our investigations showed this to be the case.

It should be mentioned that considerable variations have occurred in the rapidity with which the haemagglutinating macroglobulins appear in animals. This is in analogy with the conditions in human beings and is not surprising since it is a question of biological changes i.e. changes of the enzymatic processes in the organism provoking a new type of gammaglobulin metabolism.

Speaking of the metabolic changes studies of the NH end groups of the Rheumatoid factor

Table VI. Production of macrolobulins indistinguishable from the Rheumatoid factor (RF) by injections of an anti-RF serum or a 7 S fraction of it called IgR

Haemagglutination titre (sheep cell test) ^a after injections into rats (Sprague-Dawley) of the following materials					
	Anti-RF serum (rabbit) to rat A	Normal rabbit serum to rat B	RF neutralizing 7 S globulin called IgR to rat C	Isolated γ S globulin from human gamma globulin to rat D	Inject (0.5-0.5 ml) intraperitoneal or in the neighbourhood of the epiphyseal plate of a knee joint
1968					
Jan 15	0	0	0	0	A B C
Jan 18					A B C
Jan 30					A B C
Febr 5	1:64	0	0		
Febr 14					A B C D
Febr 15					B
April 1	1:1.5	1:8	1:64	1:8	
April 29	1:32	0	1:32	1:16	
April 30					A B C D
May 15	1:16	1:16	1:64	1:16	
Aug 19	1:512	1:16	1:64	0	
Nov 25		dead			A C D
1969					
Jan. 20	1:1.5		1:1.5	0	

^a Considered negative with a titre of 1:1.

rum of the rats. This rat serum containing the RF (or a very similar substance) gave rise to a positive sheep cell test often with high titre (i.e. up to 1:1024).

We found great and hitherto unexplained variations in the appearance of haemagglutinating macrolobulins provoked by injections of anti RF as may be seen from Tables V and VI. It should be added that rabbits reacted unfavourably to the injections of anti-RF. In order to stress the connection between the RF and the 7 S fraction of anti RF serum we have tentatively called this fraction IgR.

DISCUSSION AND CONCLUSION

It may be considered to be established that macrolobulins can be provoked through the action of bacteria. This is particularly clear as far as the haemagglutinating macrolobulins of the Rheumatoid factor type are concerned (7, 9, 16) and had been proved already in 1954-55 (Svartz). In our experience injections of *Diplostreptococcus agalactiae* constitute the best method hitherto for this purpose. At the same time arthritis is often produced. It has to be mentioned that *Diplo-*

streptococcus agalactiae is sometimes hard to differentiate from *enterococci* even on typing with specific sera. In fact we called our strains "enterococci" during the first period.

As regards the development of arthritis, many investigators have provoked joint diseases by other means than those employed by us. Advanced experiments have been performed, for instance with Freund's adjuvant (1, 2, 4). In connection with these experiments an RF like macrolobulin does not seem to have arisen.

From some earlier observations it was obvious that not only *agalactiae* but also other bacteria are able to alter the protein metabolism resulting in the formation of a haemagglutinating macrolobulin. Rheumatic fever has probably nothing to do with *agalactiae* as it is caused by haemolytic streptococci of group A. In 1953 (6) we showed that a transitory positivity of the sheep cell test is rather common in rheumatic fever. The positive sheep cell test appeared after the patients had had the disease for 4-6 weeks. In this connection it is interesting that Fudenberg and Kunkel (2) observed a positive sheep cell test in *epithymitis*.

Some years ago a strain of the group A streptococcus was received from Russell Cecil which he has been studying in collaboration with R. Lancefield. These cocci gave rise to a transient positivity in rats similar to that in rheumatic fever.

Other bacteria as well have been found to provoke a positive sheep cell test but usually with low titre.

When discussing these matters it has to be borne in mind that some investigators including our group made the mistake initially of not completely absorbing the heterophile haemagglutinins before giving the sheep cell test. This was the case in our earliest experiments with injections of cocci into rabbits. Thus it sometimes happens that the heterophile agglutinins are difficult to absorb and give rise to a strongly positive sheep cell test. This fact is now well known. Therefore an incomplete absorption of heterophile haemagglutinins is an error which hardly occurs in our days.

The question whether *Diplostreptococcus agalactiae* might be the aetiological agent of Rheumatoid arthritis (RA) has been discussed by Svartz earlier. No conclusive proofs exist that this is the case. But it is interesting that the bacteria in question are cultivated more often from the throat and stools of patients suffering from RA than from healthy individuals. This fact is of course in proof that DSA is involved in the aetiology and pathogenesis of RA. A large material would be needed for instance in order to state the difference between RA patients and healthy individuals as regards the presence of DSA in the sputum (also confirmed by V. Houba, M. D. Prague).

It is a hitherto unexplained circumstance that a haemagglutinating macroglobulin though in a rather low percentage (except in disseminated lupus) is found in rather many diseases of dissimilar aetiology. We have for instance sometimes found a positive sheep cell test in liver cirrhosis and chronic pancreatitis (13). The haemagglutinating macroglobulins in these conditions may be produced by some other types of bacteria than in RA or by viruses or toxins.

The macroglobulins giving rise to haemagglutination differ from each other in sedimentation coefficient etc. but their haemagglutinating fragments seem to be identical or very closely related. Thus the macroglobulins as such are not needed for a positive sheep cell test but as we have seen it is necessary that they contain a specific

haemagglutinating fragment (subunit). As reported before this haemagglutinating fragment has been isolated (Svartz). Like the so called γ G or IgG it is a gammaglobulin with a sedimentation constant of 6-7S but it has other biological properties. One of the most important differences is that the haemagglutinating fragment does not react with common anti γ G. It reacts strongly with anti kappa serum but it gives also a weak precipitation with anti lambda.

As pointed out above we have been able to produce a haemagglutinating macroglobulin by a method quite different from the bacterial one. After having produced an antibody against the isolated RF we injected this antiserum into rats. A haemagglutinating macroglobulin with high titre in the sheep cell test and showing the same properties as the Rheumatoid factor was produced. However this haemagglutinating macroglobulin often disappeared from the blood of the rat if the injections were not repeated. This is in contradiction to the results with bacterial injections. In the latter case the haemagglutinating macroglobulin often remains for several months without new injections (Tables I-III). The reason for this dissimilarity is possibly that when bacteria are injected they usually provoke a more or less pronounced continuous inflammatory process in the connective tissue which is easy to verify microscopically. Injections of gammaglobulins do not provoke any distinct microscopical changes in the connective tissue.

The fraction of the anti RF serum which reacts with RF has been isolated. It is a 7S globulin but not identical with the common γ G (IgG). While the common 7S is able to neutralize the RF only to a certain extent (more pronounced when the material is aggregated) the anti RF serum or its active principle neutralizes RF completely. This RF neutralizing fragment represents a new experience. For reasons which have been mentioned above and in earlier paper (14) we have for the present called this RF neutralizing fragment IgR. The study of this agent is our special concern at present.

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SCLEROSIS OF THE ARTERIES OF THE EXTREMITIES IN RHEUMATOID ARTHRITIS

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In a series of 370 patients with rheumatoid arthritis and 438 controls radiological vascular changes were observed in 15.4% of the former and 3.4% of the latter. The high rate of systemic manifestations in the rheumatoid patients showing radiological changes may be interpreted as evidence in favour of the view that arthritis is an inducing factor in atherosclerosis.

In the last few years the various systemic manifestations of rheumatoid arthritis have attracted increasing interest. Changes have been observed in many organs. Vascular lesions which are the most common were described at the end of the 1950s by B. Cruickshank. This investigator found evidence of arteritis at autopsy in 25% of a series comprising 72 cases of rheumatoid arthritis (5).

Vascular lesions are mainly encountered in small arteries, arterioles and venules (3, 11, 12). The arterial lesions may cause unspecific symptoms such as cold hands and Raynaud's phenomenon. Even cutaneous ulceration in the legs may occur (8). Acute arteritis in the mesenteric vessels may lead to intestinal ulcers and perforation (1, 6). More typical of rheumatoid arthritis is the occurrence of punctate necroses in the finger tips and slight haemorrhages in the skin folds (4).

MATERIAL AND METHOD

The series consists of 370 patients with rheumatoid arthritis and 438 controls. The controls were selected from the patients at the Orthopaedic Hospital of the Heinola Foundation. The controls showed no systemic disease and no severe disabling lesions. Patients who had been immobilized for long periods were not included.

Ordinary radiographs were taken of the blood vessels in the hands, arms, feet and legs. The radiological

method and the film quality were standardized. All radiographs were examined by one and the same person, and only obvious vascular shadows were taken into account. Patients with diabetes mellitus or disturbances of the lipid metabolism were omitted from the series.

RESULTS

Affected arteries were observed in 57/370 (about 15.4%) of the patients with rheumatoid arthritis and in 15/438 (about 3.4%) of the controls (Figs 1-2). A marked difference was observed in respect of the age distribution.

Calcium deposits were mainly encountered in the medium sized arteries of the lower extremities, e.g. the dorsal artery of the foot and the posterior tibial artery and their branches. In some cases calcium deposits were observed in the radial and ulnar arteries and the minor arteries of the hand (Figs 3-4).

Of the patients showing radiological changes 21 exhibited systemic manifestations, i.e. renal, cutaneous, cardiac or pulmonary symptoms. Five patients had malignant rheumatoid arthritis. The duration of the illness was under five years in 18 cases with radiological changes and in these an obvious tendency towards high Waaler-Rose titres was noted. All patients except one showed satisfactory mobility.

DISCUSSION

In this study the ratio of arterial changes was significantly higher in patients with rheumatoid arthritis than in subjects not showing rheumatic disease. In addition the lesions in question were found to develop earlier in life in the former

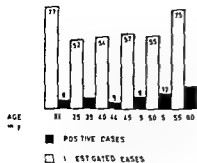


Fig 1 Radiological vascular changes in 370 patients with rheumatoid arthritis 57 positive cases about 15.4

The association of systemic manifestations and radiological vascular changes was striking. This argues in favour of the view that the vascular changes were a consequence of rheumatoid arthritis. It appears that the following three categories of arteritis may be distinguished.

(a) Subacute lesions in small arteries of muscles such as have been described by Sokoloff et al (13) and similar vascular lesions in the heart muscles and nerve sheaths as described by Cruickshank (5). This subacute arteritis which is characterized by the accumulation of lymphocytes and histiocytes in the vascular wall but is not associated with necrosis, fibrinoid degeneration or aneurysm formation was considered by Cruickshank as typical of rheumatoid arthritis. This view has not been confirmed by later investigations, however.

(b) Grave lesions resembling polyarteritis nodosa, mainly encountered in the major vessels of

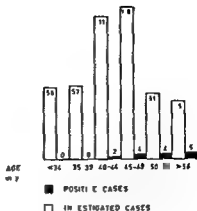


Fig 2 Radiological vascular changes in 438 control patients 15 positive cases about 3.4%

the kidneys, liver, adrenals, testes and central nervous system. Such lesions have been described by e.g. Graef et al, Levin et al, Aronoff et al and Schmid et al (2, 7, 9, 10). Microscopically acute inflammation, medial muscle cell death and fibrinoid degeneration are seen in these cases.

(c) Progressive and obliterating endarteritis in the arteries of the fingers as described by Bywaters (4). This category has very few features in common with type b.

The question of whether arteritis in patients with rheumatoid arthritis induces calcium deposition is open and cannot be answered on the basis of this study. It is also possible that the lesions in question are due to atherosclerosis. In our opinion this is a less convincing explanation con-



Fig 3 Radiograph of a 34-year-old woman with rheumatoid arthritis which began in 1961. Note marked sclerosis of distal radius of the foot.

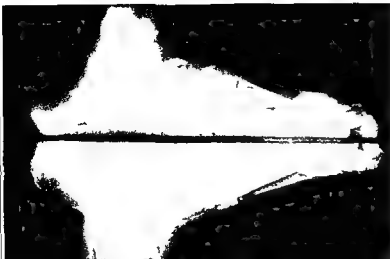


Fig 4 Radiograph of a 38 year old man with rheumatoid arthritis which began in 1955. Note marked sclerosis of dorsal artery of the foot and posterior tibial artery

sidering the strikingly high rate of positive cases in the younger age groups in the present series. Immobilisation may be thought to predispose to early atherosclerosis. In this series no correlation was observed between immobilisation and vascular changes. On the other hand a positive correlation was demonstrated between arterial changes and other systemic manifestations.

It seems obvious that rheumatoid arthritis causes connective tissue disturbances which either directly or via arteritis predispose to degenerative changes of the vascular wall resulting in fibrosis and calcium deposition which is visible on ordinary radiographs.

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Congress Announcements

Le XVI Congrès National de la Tuberculose et des Maladies Respiratoires se tiendra à Bordeaux du 2 au 5 Avril 1970

Information : Secrétariat du Congrès Laboratoire d'Hygiène Faculté de Médecine Place de la Victoire 33 Bordeaux France

The Second International Air Pollution Conference will be held in Washington D C during December 1970

Program Co-chairman Professor Arthur C Stern
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QUANTITATIVE LIPID ANALYSIS OF HUMAN LIVER NEEDLE BIOPSY SPECIMENS

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Abstract A method is described for quantitative analysis of different lipid fractions from ordinary percutaneous liver biopsy specimens. In a series of 37 liver biopsies a significant correlation was found between free and esterified cholesterol and between triglycerides free and particularly esterified cholesterol respectively.

Determination of liver lipids under basal conditions and during different phases of therapy of hyperlipidemias could give valuable information as far as fatty liver, the kinetics of serum lipids and the mode of action of therapeutic measures are concerned. Yet the number of studies dealing with lipid analyses of needle biopsy specimens is limited (2, 3, 5) and furthermore data on detailed determinations of various lipid classes are lacking mainly because of the small amount of tissue available. The combination of thin layer chromatography (TLC) and gas liquid chromatography (GLC) techniques for analysis of small amounts of tissue lipids appears to be sensitive enough and in addition offers the possibility of determining fatty acid composition of different lipid classes. Application of this procedure to lipid analyses of liver biopsy specimens is reported in the present paper.

METHODS

Percutaneous liver biopsies were performed by the Menghini technique (8). Additional samples were taken at autopsy or from rats maintained on an ordinary laboratory diet. Immediately after performance of the biopsy the specimen was placed in a preweighed glass stoppered tube and weighed. The tissue was then homogenized by a Potter Elvehjem type apparatus in chloroform-methanol (1) to which C-cholesterol (free and esterified) and β -sitosterol (free and esterified) were added for recovery tests. The chloroform phase was evaporated and lipids were separated by TLC on silica gel G (6) developing chroma-

toplates with ethyl ether-heptane and acetic acid (40:10:1). Cholesterol esters, triglycerides, FFA, cholesterol and phosphatides could be easily recovered from the plate for qualitative and quantitative analysis after spraying with rhodamine. Cholesterol and cholesterol esters (saponified in alkaline ethanolic solution) were determined as their TMS-ethers by GLC using 5 α -cholestan as an internal standard (9). Correction for the losses taking place during the procedure was made by the recovery of either C-cholesterol or β -sitosterol. Triglycerides were measured by the carboxyl ester reaction (10) or by GLC of fatty acid methyl esters (5) prepared by methylating triglyceride fatty acids with methanol-HCl after preceding saponification. Trimargarone was used as an internal standard. The loss of triglycerides during the isolation procedure was assumed to be the same as that of cholesterol fractions. Phosphatides were determined colorimetrically (1) after digestion of the phosphatide-containing silica gel area of the chromatoplate or by the carboxyl ester reaction after methylation of fatty acids released from phosphatides by saponification. GLC of fatty acid methyl esters was carried out using a 15% diethylene glycol adipate column. The water phase of the homogenization mixture was used for determination of protein (4).

RESULTS AND DISCUSSION

The recovery of cholesterol and cholesterol esters was 90-98% according to the recovery of added radioactivity or β -sitosterol. Although the liver tissue contained trace amounts of endogenous plant sterols, a mixture of free and esterified β -sitosterol in relatively large amounts appeared to be an ideal internal standard because this sterol behaves like cholesterol and could be determined in the same GLC run as cholesterol. Table I shows that although the weight of the liver pieces from a cadaver and a rat ranged in two series of six determinations from 4 mg to 41 mg and from 4.5 mg to 19.8 mg respectively, the standard deviation of the mean for free and esterified cholesterol and for triglycerides was relatively small.

Table 1 Replicability of cholesterol and triglyceride determination from liver of a human cadaver and a rat

Mean \pm standard deviation (s.d.) of six determinations

Source	Range of tissue weights (mg)	Cholesterol		Tri glycerides (g/100 g)
		Free (mg/100 g)	Ester (mg/100 g)	
Human	4-41	233 \pm 21	48 \pm 4.0	0.74 \pm 0.02
Rat	4.5-19.8	209 \pm 18	48 \pm 4.9	0.63 \pm 0.06

Thus the methodological error of the entire procedure including weighings after correction of the recovery is within the limits of $\pm 10\%$ even in the case of small tissue pieces. Similar results were obtained for phosphatides. Determination of triglycerides appeared to be more accurate by GLC than by color reaction particularly in small tissue specimens with low triglyceride content. Variation of the results was smaller on the basis of wet weight than of the protein content.

In a series of 37 liver biopsies carried out mostly on obese and hyperlipidemic subjects there was a significant correlation between free and esterified cholesterol ($r=0.75$). In addition as illustrated

in Fig. 1 liver free and particularly esterified cholesterol showed a significant correlation to triglycerides ($r=0.57$ and 0.70 respectively) indicating that the fatty liver contains also increased amounts of cholesterol. In agreement with other studies on liver needle biopsies (2, 3, 5) and biopsies taken by surgery (7) triglyceride concentration shows a wide range of variation being almost 100 fold in the present material (0.26-21.6 g/100 g). Most of the values (Fig. 1) are below 5 g/100 g which appeared to be the limit for the microscopic detection of fatty infiltration. Free and esterified cholesterol which have not been earlier determined separately from liver biopsies showed less marked variation being for free cholesterol only about 4 fold (142-620 mg/100 g) and for esterified cholesterol about 10 fold (30-378 mg/100 g).

ACKNOWLEDGEMENTS

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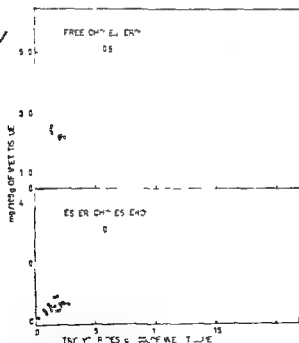


Fig. 1 Correlation of free and esterified cholesterol to triglycerides in liver needle biopsy specimens of 37 human subjects.

HERPES SIMPLEX HEPATITIS IN AN ADULT

A Case Report with Virological and Electron Microscopical Examination at Autopsy

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Abstract A case report is given of a man with bronchial asthma who fell ill in a stomatitis. He was treated initially with antibiotics and corticosteroids. Haematemesis, icterus, jaundice, anuria and circulatory insufficiency developed and the patient died. Autopsy revealed hepatitis erosive gastritis with gastrointestinal bleeding and sick kidneys. Histologically the hepatitis was consistent with a herpes simplex hepatitis. Herpes simplex virus was isolated from the liver.

Almost all cases of herpes simplex hepatitis described have been encountered in newborns with generalized herpes infection (for references see 5). In older children there is evidence of infection of the liver only in a few cases (1, 8, 9). To our knowledge herpes simplex hepatitis has not been seen in adults.

We have encountered a case of herpes simplex hepatitis in an adult with a fatal outcome. Virological and electron microscopical examinations performed on material obtained at autopsy in the present paper will give a brief description of the case.

CASE REPORT

The patient was a male factory leader born in 1907 with bronchial asthma since 1970, possibly of infectious origin. In 1959 and 1960 he was treated with Acton[®] (Ferring A/CII) 10 IU every three days and adrenalin spray. From the end of 1960 Acton was changed to Kenacort[®] Squibb (fluocyprednisolone) 4 mg a day which was taken rather regularly.

On November 7, 1967, the patient developed pains in the throat, headache and a rectal temperature of about 39°C. He had no asthma symptoms and took no Kenacort.

He was treated with Tetracena Novum[®] Astra (tetracycline) 0.25 g \times 4 for one day followed by Dokta-

cillin[®] tablets Astra (ampicillin) 0.5 g \times 4. The temperature remained elevated and he was admitted to the County Hospital on November 10. He was then in a fairly good condition. The tongue was white and in the tonsillar regions there were white papules surrounded by red zones. The treatment with Doktacillin continued with the same dosage. He was also given Kenacort 2 mg \times 4 from November 13, 4 mg \times 2 and Bamyll[®] Hassle (acid acetylsalicyl) 0.5 g calc carb 0.15 g and acid citr 25 mg per tablet) 2 tablets \times 4. The temperature fell to about 38°C. Due to his aching stomatitis he ate almost nothing and gradually deteriorated.

On November 17 a rather large haematemesis occurred followed by bloody diarrhoea. An enlarged liver was noticed. Urine became sparse and did not increase despite treatment with 300 ml Mannidex[®] Pharmacia (15% mannitol). He was also given 1000 ml Inverdex[®] Pharmacia (sacchar invert 100 g, NaCl steril ad 1000 ml).

On November 18 the patient was transferred to the Department of Infectious Diseases, University Hospital, Uppsala. He was deep-breathing and his tongue and most of the oral cavity were covered with yellow crusts. The systolic blood pressure was 125 mm Hg. The liver was slightly enlarged and tender. There were still small amounts of urine. His electrolyte balance was greatly disturbed (see Laboratory data). He had repeated bloody diarrhoea and the systolic blood pressure fell to 80-85 mm Hg. The temperature was around 38°C. On this day he was given Inj Doktacillin[®] 0.5 g \times 3, Inj Solu Glyc[®] Erco (hydrocortison) 300 mg, 1000 ml Inverdex 50 ml Mannidex 450 ml blood Inj Calcium Sandoz[®] totally corresponding to 90 mg Ca, 15 g Resonium[®] Winthrop (sodiumpolystyrene sulfonate) and Inj Aramin[®] MSD 50 mg in 500 ml Inverdex.

On November 19 the urine secretion totally ceased, the blood pressure remained low and the patient died.

Laboratory Data

Nov 11 Hb 150 g, 100 ml. White blood cell count (WBC) 4300/mm³ with 10% polymorphonuclear neutrophils, 1% basophils, 16% lymphocytes and 2% mono-

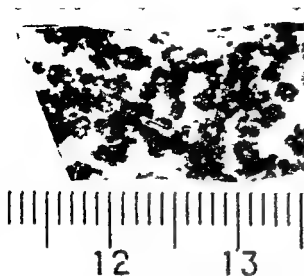


Fig 1 The surface and the interior of the liver is dotted with haemorrhagic necroses. Measurements shown in cm.

cytes Serum Na 131 mEq/l (normal 134-146 mEq/l) serum K 3.6 mEq/l (normal 3.6-4.6 mEq/l) serum Ca 4.8 mEq/l (normal 4.0-5.2 mEq/l) serum Cl 95 mEq/l (normal 97-108 mEq/l) serum protein 6.6 g/100 ml (normal 7.1-8.1 g/100 ml). The urine contained albumin but only few cells. Serum creatinine 1.5 mg/100 ml (normal 0.6-1.2 mg/100 ml).

Nov 14 Serum bilirubin 0.6 mg/100 ml (normal 0.2-1.0 mg/100 ml) thymol turbidity test extinction value 0.05 (normal < 0.1) serum alkaline phosphatase 6 Bessey Lowry units/100 ml (normal 0.9-2.5 units/100 ml) serum glutamic pyruvic transaminase (SGPT) 400 units (normal < 35). Cold agglutination test neg.

Nov 16 Cerebrospinal fluid total globulin 76 mg/100 ml (normal 70-90 mg/100 ml) Masut neg. No cells. Serum creatinine 4.6 mg/100 ml.

Nov 17 Thrombocytes 103 000.

Nov 18 Hb 15.4 g/100 ml Haematocrit 46% WBC 4400 with 87.5% polymorphonuclear neutrophils, 0.5% basophils, 4% lymphocytes, 3.5% monocytes, 3.5% plasma cells and 1% metamyelocytes. Thrombocytes 88 000 ESR 17 mm/1 h. Serum creatinine 10.3 mg/100 ml serum bilirubin 0 mg/100 ml thymol turbidity test 1 MacLagan units (normal < 3) serum alkaline phosphatase 8.7 units/100 ml serum glutamic oxaloacetic transaminase (SGOT) 6.90 units (normal < 35) SGPT 1800 units. Serum Na 171 mEq/l serum K 6.0 mEq/l serum Ca 3.9 mEq/l serum Cl 9.0 mEq/l plasma HCO₃ 19 mEq/l (normal 18.5-24.5 mEq/l).

Nov 19 Hb 13.1 g/100 ml serum creatinine 14.0 mg/100 ml serum Na 110 mEq/l serum K 5.1 mEq/l serum Ca 2.6 mEq/l serum Cl 80 mEq/l serum protein 5.1 g/100 ml.

Candida albicans was cultured from urine and faeces on Nov 18.

Autopsy

Macroscopical examination

Autopsy was performed 46 hours after death. Situs in versus was found but no other malformations.

On the skin no vesicles or crusts were seen. On the tongue the palates and the buccae there were several white papules 3-5 mm in diameter often crusted or ulcerated.

The lungs showed slight emphysema but no bronchitis or bronchiectasis. They were of normal size. The heart weighed 530 g but there was no cor pulmonale.

The liver was studded with haemorrhagic necroses, 3-6 mm large over the outer and cut surfaces (Fig. 1). It weighed 2050 g and was very swollen.

The spleen was heavily congested and weighed 715 g. The kidneys were slightly swollen and had a pale cortex. They weighed 205 and 190 g. In the pelvis of the right kidney there was a coral stone partially obstructing one calyx. The urine bladder showed slight cystitis. The prostate was nodulated and of benign appearance.

The stomach and the small intestines had bloody con-



Fig 2 Histological section of the liver. Necrotic areas are seen in the upper and lower part of the picture. There is a slight fatty change in the preserved parenchyma. Haematoxylin-eosin. 90.

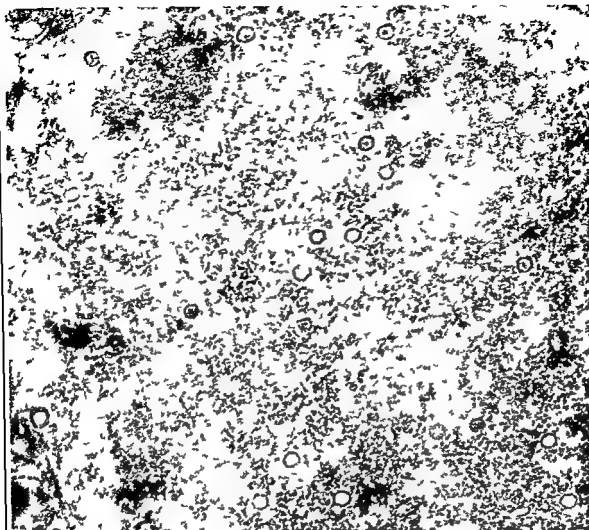


Fig 3 Electron micrograph of section of liver cell nucleus. Virus particles with hollow and dense cores are seen. Uranyl acetate lead citrate $\times 47,000$.

tents. There were several small shallow erosions in the distal part of the stomach. No other relevant findings were made.

gross pathological examination

The buccae showed shallow ulcers with fibrin and polymorphonuclear leucocytes. No giant cells or inclusion bodies were found.

The liver showed a large number of often confluent hemorrhagic necroses (Fig 2). They were situated in all parts of the lobules and often covered more than one lobule. Many erythrocytes and a moderate and varying number of polymorphonuclears were found within the necroses. The demarcation towards the vital tissue was rather sharp. In this zone occasional liver parenchymal cells were found with intranuclear inclusion bodies. In the peripheral parts of the necrotic areas necrotic nuclei

taking the same colour as the inclusion bodies were often seen. A few liver cells had 2-3 nuclei. Occasional parenchymal cells had a ballooned appearance. The preserved part of the liver parenchyma showed a slight fatty change.

The stomach showed erosions. It was too autolytic for reliable examination. The kidneys were autolytic. A large number of hyaline and haemoglobin casts were found.

The other organs examined (heart, lungs, spleen, pancreas and intestine) showed no findings of interest.

Electron microscopy of the liver

The tissues had been fixed in 10% formaldehyde for a few days. They were postfixed in glutaraldehyde and osmium acid according to Hubner (1965) and embedded in Epon. Sections were cut with an LKB ultramicrotome, stained with uranyl acetate/lead citrate and examined in a Siemens Elmiskop I electron microscope.

Virus particles were found in a few liver cell nuclei often in large amounts. The particles consisted of a ring with hollow or dense cores (Fig 3). In the cytoplasm virus-like particles were only occasionally encountered. Their viral nature was questionable. No typically enveloped particles were seen.

Virological Examination

For virus isolation supernatants of 0.2 homogenates of liver, spleen, kidney, lung and blood clot from the heart were prepared and inoculated into cultures of BSC-1 cells (a stable line of monkey kidney cells). No cytopathic changes were seen after the inoculation of the specimens from the spleen, kidney, lung or blood clot. The liver specimen however caused cytopathic changes characteristic of herpes simplex virus. The isolate was typed by neutralization with antiserum to a known strain of herpes simplex virus (strain E263 isolated from a patient with stomatitis).

Serum, taken at autopsy, was tested for neutralizing antibodies using 100 TCD₅₀ of virus. The titre against the strain E263 as well as the strain isolated was 1/16. The serum could not be used in the complement fixation test due to its haemolytic effect.

Summary of Autopsy and Virological Findings

Stomatitis. Herpes simplex hepatitis. "Shock kidney". Erosive gastritis with gastro-intestinal bleeding.

DISCUSSION

The 60-year-old patient had thus had bronchial asthma for 47 years which had been treated with ACTH and corticosteroids for the last eight years. He developed a stomatitis, the aetiology of which was not known.

He was treated with inter alia antibiotics and corticosteroids but deteriorated rapidly. Haematemesis, melena, anuria and jaundice developed and the blood pressure was falling. The patient died twelve days after the beginning of the illness.

Autopsy revealed stomatitis, multiple haemorrhagic necroses in the liver, erosive gastritis with large gastrointestinal bleeding and shock kidneys. In the liver intranuclear inclusion bodies were found under the light microscope and virus particles in the electron microscope. Herpes simplex virus was isolated from the liver. The macroscopical, light microscopical and electron microscopical picture of the liver was consistent with that described for infant with herpes simplex hepa-

titis (4). However it was not a giant cell hepatitis, which has been described in some cases of herpes simplex hepatitis in newborns (3).

The hepatitis might have been the result of a primary or a recurrent infection with herpes simplex virus. At the age of the patient a primary infection is uncommon but cannot be excluded. If the stomatitis was caused by herpes simplex virus a primary infection is even the most probable alternative. It is argued that herpes simplex stomatitis seldom if ever recurs (7).

The development of the hepatitis might have been due to the corticosteroid treatment as this may be deleterious in viral infections. The erosive gastritis may also have been a sequel of the corticosteroid treatment but an effect of the salicylates (Damyll) may be considered (6). Liver injury might also have been a factor in the development of the erosive gastritis as increased levels of SGPT and serum alkaline phosphatases were present on November 14, three days before manifest signs of gastrointestinal bleedings. The possibility also exists that the gastritis was caused by the virus. Lesions in the stomach have been found in cases of generalized infection with herpes simplex virus (9). In this case the stomach was too autolytic for reliable examination.

The voluminous gastrointestinal bleedings and the extensive liver necroses were considered to be responsible for the falling blood pressure and consequent anuria. It was impossible to decide which of these two factors was the predominating one for the fatal outcome. A pure bleeding shock should have been associated with a lower haemoglobin value.

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QUINIDINE PRIOR TO DIRECT CURRENT COUNTERSHOCK —A MEDICAL HAZARD

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Abstract The author has previously discussed a possible relationship between post conversion arrhythmias and quinidine administered prior to a countershock. A further evaluation of the suspected relationship is now possible after a more extensive follow-up. Evidence is provided that quinidine as well as digitalis should be avoided immediately before direct current conversion. The reason is the highly increased risk of post conversion arrhythmias.

RESULTS AND COMMENTS

The occurrence of post conversion arrhythmia among the different groups is presented in Table I. Complete absence of ventricular tachycardia and fibrillation is noted in the group in which digitalis was discontinued and quinidine not administered prior to the shock.

It is seen in the table that many patients in group 1 underwent repeated conversions. The ratio of the number of conversions to the number of patients is 1.5. The corresponding ratio in group 3 is 2.2. In other words there were more reconversions in the latter group. Thus in spite of the fact that late recurrence of fibrillation is much more common in group 3 (i.e. group 3 represents a worse cardiac state than groups 1 and 2) the immediate post conversion complications discussed do not appear.

The results should be considered in the light of this fact which further supports the assumption of a relationship between quinidine prior to the shock and an increased occurrence of post conversion arrhythmia.

MATERIAL AND METHODS

Our series of patients with atrial fibrillation treated by DC shock has been presented earlier (3, 4).

The method of conversion and of anesthesia has already been described (3, 4, 6). With regard to drugs and the countershock procedure the rules have changed in our clinic since we started to use the DC technique in

Since January 1966 we have discontinued digitalis prior to the shock (3). Since September 1966 we have not given quinidine until after the shock. Before then the patients had a serum level between 4-7 mg/l at the time of the countershock, considered as normal maintenance according to our method (8). Sixty-six patients in 1967-1968 received 0.5 g procainamide 2-3 hours before the shock. These patients were included in clinical investigations (1, 7).

Thus the material is divided into three main groups according to the different schedules of premedication (1).

DISCUSSION

The procedure of discontinuing digitalis therapy before the DC shock has been well supported by clinical reports as well as by animal studies (10, 11). Lown tested the energy levels necessary to start a ventricular tachycardia or fibrillation in a certain number of dogs (10). After having titrated the digitalis toxicity level for these dogs he repeated the experiment. He found a very marked decrease of the energy necessary to give the dogs ventricular tachycardia or fibrillation.

Table I Number of post conversion ventricular arrhythmias during different drug schedules at the time of countershock

Drug regimen prior to shock	Time	No of pats.	No of conversions	Ventricular tachycardia and or fibrillation
1 Digitalis and quinidine	Aug. 1963 - Jan. 1966	160	234	4
2 Quinidine alone	Jan. 1966 - Sept. 1966	29	38	4 ^b
3 No therapy	Sept. 1966 -	113	243	—

^a One patient did not have quinidine

^b One patient did not have quinidine. This and one other patient were converted at the end of 1965 but did not receive drugs during the last 2 days prior to the countershock.

when they were intoxicated by digitalis. The reason for discontinuing digitalis prior to shock treatment would then be to minimize the number of undetected digitalis intoxications. The number of such patients might easily be underestimated owing to the common use of diuretics with a potassium lowering effect potentiating digitalis. The principle of discontinuing digitalis prior to a DC conversion is now generally accepted.

With regard to quinidine however the opinions differ (3, 7, 9, 12, 13). Rossi et al. discuss the problem of quinidine administration at the time of conversion in a recent report (13). They conclude that quinidine prior to shock has several advantages for example to avoid early relapse into atrial fibrillation to make the procedure safer, as regards complicating arrhythmias and finally a few patients are already converted by quinidine.

The number of patients in their study was small. There were 24 patients in the quinidine group and 23 in the control group.

In the review of the results they graded the post conversion arrhythmias according to severity from zero to four. Grade zero was no supra- or ventricular ectopic beat and grade four ventricular bigeminy and ventricular tachycardia. There were three patients in the control group belonging to grade four and one in the quinidine group. No information is given as to which case or cases had ventricular tachycardia or ventricular bigeminy and nothing is said as to the seriousness of the arrhythmia. Further the difference found is not significant (Fisher's *t* test).

Their results with regard to ventricular ectopic beats were in favor of the quinidine group but with regard to nodal rhythm in favor of the control group. The numbers were small and the statistical analysis questionable however.

There are some further objections to the study of Rossi et al. In only 15 patients were the serum levels of quinidine determined and in this group the concentration ranged between 0.36-4.19 m/l. The mean was 2.2 mg/l. If these serum determinations are representative of the entire group the usually accepted maintenance level was not reached in their series. Therefore their results are inconclusive as to the comparison between a group of patients on maintenance quinidine therapy at the time of conversion and a group without the therapy.

Some other investigators who do not give quinidine prior to the shock have experienced no ventricular tachycardia or fibrillation (7, 9). In a recent report by Radford and Evans no instance of these arrhythmias occurred in 34 patients immediately post conversion (12). Quinidine in a dose of 0.4 g had been given three hours before the shock, which should only result in a low serum level.

Some interesting case studies by Castellanos et al. also support the opinion expressed in the present paper (5).

If quinidine is not started until after the conversion there might be a few early relapses into atrial fibrillation. These patients however still have a poor prognosis as regards maintenance of the sinus rhythm (4, 12). The possibility of avoiding early relapses does not justify the risk of increasing post conversion arrhythmia through drugs administered prior to the shock treatment.

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THE RENAL METABOLISM OF CITRIC ACID IN RENAL TUBULAR ACIDOSIS

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Abstract The renal metabolism of citrate has been studied in a patient with renal tubular acidosis. In accordance with previous studies, low citrate excretion was found during acidosis. The decreased clearance of citrate was related to an apparently high tubular reabsorption of citrate probably due to decreased formation and secretion of citrate from the tubules. The amount of citrate metabolized in the kidneys was in the normal range. Low normal citrate excretion was found after correction of the acidosis. Correction of hypokalemia had no effect on citrate excretion.

The kidney participates in the regulation of acid-base balance by reabsorbing the filtered bicarbonate and by regenerating bicarbonate (7). The latter process depends on the formation of titratable acid and the secretion of ammonia. The reabsorption and regeneration of bicarbonate are mediated by a common mechanism involving secretion of hydrogen ions in exchange for sodium both in the proximal and in the distal tubules. The renal capacity for regeneration of bicarbonate is normally sufficient to restore the amount of bicarbonate decomposed in plasma by invasion of strong acids formed by metabolic processes in the organism. If the regeneration of bicarbonate is less than the acid formed, metabolic acidosis will ensue. In such a condition the pH in urine will be low. In renal tubular acidosis (RTA) the pH in urine is high despite metabolic acidosis.

Different pathogenetic factors for RTA have been discussed (8-10) among others a deficiency of the enzyme carbonic dehydratase. This defect will produce hyperchloremic acidosis which characterizes RTA. Blocking of carbonic dehydratase produces a decrease in bicarbonate and increased leakage of bicarbonate. Different from the pattern in RTA where bicarbonate T_m is normal and the spillage of bicarbonate is diminutive (10). In addition normal ac-

tivity of carbonic dehydratase in renal tissue has been found (12). A different distribution of carbonic dehydratase in the nephron might possibly cause a similar defect to that in RTA. There is however no evidence to support this assumption.

The disorder may be an inability to establish a high gradient of hydrogen ions between plasma and urine. The reason for this defect in acidifying the urine is unknown. The hypothesis of a back diffusion of hydrogen ions in the distal tubules due to permeability changes (7) is in our opinion too simple and bypasses the cellular mechanisms involved. The urinary excretion of citrate is decreased in RTA (6, 11). Milne et al (5) proposed that low excretion of citrate is related to intracellular acidosis. Studies by Morrissey et al (6) lend support to this hypothesis. After correction of the acidosis with bicarbonate a normal citrate excretion was seen by these authors. In contrast Wrong (2) is of the opinion that decreased excretion of citrate is due to a deficiency of some enzyme essential for the normal functioning of the Krebs cycle. This enzyme defect might be responsible both for the low citrate excretion and the inability to acidify the urine. After correction of the acidosis he observed an increase of citrate excretion but not to normal levels.

Inhibition of carbonic dehydratase produces reduced citrate excretion in urine, depression of the renal metabolism of citrate while tubular reabsorption of citrate is normal or slightly depressed (1). Acetazolamide apparently also influences the aerobic renal metabolism with reduction of renal oxygen consumption (1). The present studies were undertaken to elucidate the renal metabolism of citric acid in RTA to give additional information on the pathogenesis of this disorder.

Table 1 Renal function studies and renal clearance metabolism and tubular reabsorption of citrate during metabolic acidosis of varying degree in a case of renal tubular acidosis

	Renal vein catheterisations						Conventional clearance measurement	
	First examination			Second examination			Third examination	
Electrolyte and acid/base state pH		7.30			7.35		7.40	
pCO ₂ (mm Hg) St bicarb	37		18	27		17	36	22
Na K Cl (mEq/l)	130	2.6	101	140	4.0	119	—	—
Citrate concentration in blood (mg/100 ml plasma)		2.32			3.06			4.08
		(art blood)			(art blood)			(ven blood)
Arterio venous difference (mg/100 ml plasma)		0.76			0.77			
Metabolized in kidney (mg/min)		3.00			2.90			
Filtered through glomeruli (mg/min)		1.77			2.57			
Excreted in urine (mg/min)		0.10			0.32			1.00
Inulin clearance (ml/min)		76			84			
Renal plasma flow (ml/min)		389			417			
Citrate clearance (ml/min)		4.48			10.58			4.51
Tubular reabsorption of citrate (mg/min)		1.67			2.25			
Percentage of filtered citrate reabsorbed		95			87			

METHODS

The patient studied was a 17 year-old female who had suffered from RTA from the age of 4 months and had revealed nephrocalcinosis growth retardation systemic acidosis and hypokalemia.

The first examination was performed while the patient was acidotic. Eight months later a second study was undertaken after the patient had been treated more vigorously with bicarbonate but still was slightly acidotic. The first two studies were done by renal vein catheterization. In a third examination, after normal acid base balance was restored conventional clearance of citrate was determined.

Citric acid was determined by the pentabrom acetone method. The renal citrate utilisation was calculated in accordance with Herndon *et al* (4). Methods used for the determination of glomerular filtration rate (GFR) and renal plasma flow (RPF) were as previously described (1).

RESULTS

The results are presented in Table 1 and are average values from 2-3 periods. The average arterial citrate level varied between 2.32 and 3.06 mg/100 ml which is in the normal range. Clearance of citrate was significantly decreased during the first two studies. After correction of the acidosis the citrate clearance was higher. The amount of citrate utilized by the kidney was in the normal range while tubular reabsorption of citrate was significantly above the upper normal limit.

The renal metabolism measured as oxygen con-

sumption was normal in this patient indicating a normal metabolism of citric acid (1).

Normalisation of citrate excretion after correction of the acidosis supports the view that changes in intracellular pH are responsible for the altered urinary excretion of citrate. Changes in intracellular pH will in our opinion influence the formation and tubular secretion of citrate. It has been suggested that potassium depletion might produce low citrate excretion. Morrissey *et al* (6) found no increase in citrate excretion after potassium administration in RTA with hypokalemia. They observed increased citrate excretion in the lower limit of normal range after correction of the acidosis and hypokalemia with KHCO₃. These results indicate that low citrate excretion is not related to potassium depletion *per se*. Our observations are in accordance with the results of Morrissey *et al*. During the second examination when the excretion of citrate was decreased and she was still acidotic the hypokalemia was corrected.

Recently changes in the citrate content in renal tissue have been demonstrated in connection with different metabolic disturbances (9). Further examination of the renal metabolic defect responsible for decreased formation and tubular secretion of citrate in RTA necessitates renal tissue analysis of citrate content.

The low citrate excretion may be an important

factor for the formation of nephrocalcinosis in our patient. Nephrocalcinosis is also seen in other acidotic states for example chronic carbonic dehydratase inhibition (3). The citrate forms with Ca^{++} a chelate complex which is soluble but dissociates poorly and thus binds Ca^{++} ions which otherwise could precipitate from the urine.

COMMENTS

Citrate clearance is normally $35 \text{ ml/min} \pm 14$ (1). Citrate clearance in this patient varied from a significantly decreased value in acidosis to a low value within the normal range after correction of the acidosis. The reason for decreased citrate excretion was apparently increased tubular reabsorption of citrate. About 70% of filtered citrate is normally reabsorbed (1). During acidosis the tubular reabsorption of citrate varied between 95 and 87%. This apparently increased reabsorption of citrate may be the result of

- (a) reduced tubular secretion of citrate
- (b) increased oxidation of filtered citrate by tubular cells

Due to tubular secretion the calculated values of tubular reabsorption of filtered citrate in normal persons may be estimated too low.

The amount of citrate metabolized was within the normal range and significantly higher than the amount reabsorbed in accordance with observations in normal persons (1). Thus we have observed an apparently increased reabsorption of citrate when the patient was acidotic while the amount metabolized was in the normal range. This could indicate that formation and tubular secretion of citrate are reduced in RTA.

The pattern of tubular reabsorption and metabolism of citric acid in RTA is not in accordance with the observations after inhibition of carbonic dehydratase. Acetazolamide produces a reduction of the amount of citrate metabolized while tubular reabsorption is in the normal range. Our results support the opinion that carbonic dehydratase activity is normal in RTA.

ACKNOWLEDGEMENT

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RIGHT ATRIAL MYXOMA

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Abstract A review of the symptomatology of right atrial myxoma is given, based on 33 cases from the literature and on two cases studied by us.

This diagnosis should be considered in any patient with isolated right heart failure without pulmonary congestion, pulmonary disease or pulmonary stenosis.

A tricuspid murmur intermittent cardiac or cerebral symptoms or radiographic demonstration of non valvular intracardiac calcifications should increase the suspicion.

Selective right atrial angiocardiology is the diagnostic method of choice. The diagnosis cannot be excluded without this examination, which always should be performed in any patient with isolated tricuspid valvular disease.

Primary tumors of the heart are rare. Strauss and Merliss (30) found a rate of 17 primary tumors per million autopsies during the period 1938 to 1942. One half of the tumors reported by Prichard (25) were myxomas. Seventy five per cent of the myxomas were located in the left atrium and about 25 % in the right atrium.

Atrial myxoma is now a curable disease. The first successful operation was performed in 1954 by Crafoord (11) under direct vision during cardiopulmonary bypass. Since then several successful operations have been reported (26).

The diagnosis of right atrial myxoma is accordingly important. It can be rendered probable by angiocardiology if only the disease is suspected. Our purpose is thus to give a review of right atrial myxoma with emphasis on the symptomatology based on 33 cases previously published (1-10, 12-24, 26-29, 31-33) and on two additional cases which we have recently encountered in this Department.

CASE REPORTS

Case 1

A 51 year-old woman was admitted in February 1967 for blebular heart disease.

There was no history of rheumatic fever. At the age of

29 years she was admitted to a local hospital with symptoms of embolism of the left lung.

Two years later at the age of 31 years she had another pulmonary embolus. Treatment with digitals was started and thereafter continued.

Ever since the last episode the patient had symptoms consisting of attacks of dyspnea at rest but also to some extent on exertion. She had the feeling of being suffocated and drawn forward during these attacks but she had never fainted. She had furthermore noticed that she was unable to lie on her left side because of dyspnea.

For the last two years the symptoms progressed and she was admitted for diagnostic investigation.

Physical examination revealed a slender woman in no distress. There was no dyspnea at rest and no cyanosis. Slight engorgement of the neck veins and venous pulsation with a big a wave was seen. The liver was palpable 4 cm below the curvature. There was no ascites or peripheral edema and no pulmonary stasis was found. The blood pressure was 100/80 mm Hg.

At auscultation of the heart a systolic murmur grade 2 (of 6) was heard at the apex and at the left lower part of the sternal border. No diastolic murmur was noticed.

The electrocardiogram showed sinus rhythm with a QRS axis of +110 degrees and peaked P waves in standard leads II and III.

The roentgenogram of the chest showed moderate cardiomegaly with dense intracardiac calcifications (Figs 1 and 2).

Laboratory tests The hemoglobin was 14.6 g per 100 ml and the sedimentation rate 5 mm/h. Electrophoresis of the serum proteins showed normal distribution. A lung scanning with ¹³¹I albumen was performed and showed reduced blood flow through the left lower lobe.

Hemodynamic investigation At right heart catheterization the catheter could only with difficulty be passed through the tricuspid ostium. The pressure in the pulmonary artery was 17/5 mm Hg in the wedge position, mean 5 mm Hg, in the right ventricle 17/0 mm Hg, and in the right atrium -1 mm Hg in mid-diastole. The cardiac index was 1.4 l per min only.

A selective angiocardiology and cineangiography with contrast injection into the right atrium was performed. A large mobile mass, a part of which was calcified, was demonstrated in the right atrium.

An operation in extracorporeal circulation was performed and a yellowish tumor was completely removed from



Fig 1 Antero-posterior roentgenogram of the chest of case 1. Note the calcifications of the right atrial myxoma.

the right atrium. The tumor was pedunculated, being attached to the margins of fossa ovalis; it filled the entire atrium and was hourglass-shaped. The part facing the tricuspid ostium was calcified, while the rest of the tumor was of brittle consistency. Histological examination showed myxoma with calcification.

Postoperatively the patient developed arrhythmias mainly consisting of intermittent atrio-ventricular block, atrial flutter and atrial fibrillation. One month after operation sinus rhythm was restored through a DC countershock. At a follow-up examination one year later she still had sinus rhythm; however she still needed diuretic treatment because of moderate right heart failure, and she had a systolic murmur grade 2 at the left lower sternal border—increasing during inspiration—and pulsation of the neck veins due to moderate tricuspid insufficiency.

Case 2

A 53-year-old man was transferred from another hospital at the end of March 1967 for diagnostic investigation of possible constriction of the heart.

He had never had rheumatic fever, pleurisy or tuberculosis and he had previously been in good health.

In August 1966 he was admitted for a few days to the local hospital because of an abnormally deep Q_m in the electrocardiogram, which was taken incidentally during a medical examination for insurance purposes. His only symptom at that time had been one episode of dyspnea on climbing stairs one month previously. Physical examination was normal except for obesity. The roentgenogram of the chest was normal. He was discharged with a diagnosis of old myocardial infarction.

Four months later he developed increasing dyspnea on exertion, a persistent unproductive cough, edema of the legs and swelling of the abdomen. He was readmitted to the local hospital in March 1967. Hypoalbuminaemia was found in addition to elevated serum bilirubin and low



Fig 2 Lateral roentgenogram of the chest of the same patient as in Fig 1. Note the posteriorly situated calcifications.

prothrombin time. The roentgenogram of the chest showed enlargement of the cardiac silhouette; the electrocardiogram showed marked low voltage, which was the main reason for the suspicion of constriction of the heart.

Physical examination revealed an obese man in no acute distress. There was slight dyspnea at rest and slight cyanosis of the ears, lips and nails. Some engorgement of the neck veins was seen, but no venous pulsation. The liver was palpable about 10 cm below the curvature and there was marked ascites. There was no peripheral edema. No pulmonary signs could be detected. The blood pressure was 130/80 mm Hg.

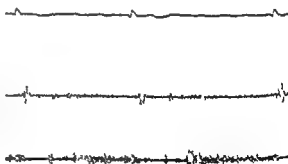


Fig 3 Phonocardiogram from the fourth left intercostal space recorded during inspiration in case 2. Note the long diastolic murmur.

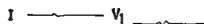
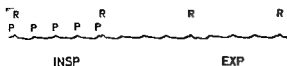


Fig 5 Electrocardiogram of our case 2

rhythm atrial fibrillation and atrial flutter with usually 4:1 block.

The roentgenogram of the chest showed a diffusely enlarged heart.

Laboratory tests The hemoglobin was 14.3 g per 100

Fig 4 Phonocardiogram of case 2 during atrial flutter with 4:1 block. The diastolic murmur during inspiration is divided into short parts synchronous with the P waves

Auscultation of the heart The heart sounds were very faint, the second heart sound being barely audible. In the fourth left intercostal space at the sternal border a peculiar murmur was heard. It was a diastolic superficial scratching medium to high frequency murmur which was only present during inspiration (grade 2) being completely inaudible during held expiration. This was found when the patient had sinus rhythm, nodal rhythm, atrial fibrillation (Fig 3).

During part of his time in hospital the patient had atrial flutter with changing block, usually 4:1 block. In this rhythm the murmur was present during the whole inspiration as short parts interrupted by brief silent intervals. The short parts of the murmur were synchronous with the P waves of the electrocardiogram (Fig 4).

The electrocardiogram showed a markedly low voltage in all leads (Fig 5). The rhythm changed—as mentioned—between sinus rhythm with wandering pacemaker, nodal



Fig 6 Selective angiocardiology with contrast injection into the right atrium of case 2. Anteroposterior (a) and lateral (b) projection.

ml, the sedimentation rate 4 mm/l hour the serum bilirubin 3.5 mg per 100 ml the thymol extinction test 2, the alkaline phosphatase 49 units per 100 ml (normal upper limit 38 units per 100 ml) the lactic acid dehydrogenase 34 units per ml (normal upper limit 24 units per ml) with the elevation being confined to isoenzyme no 1 (myocardial fraction) and the prothrombin percentage was 25 There was slight hypoalbuminemia (4.1 g per 100 ml) and hypergammaglobulinemia (2.0 g per 100 ml)

Hemodynamic investigation At right heart catheterization the pressure in the pulmonary artery was 21/14 mm Hg, in the "wedge" position mean 11 mm Hg, and in the inflow tract of the right ventricle 33/7 mm Hg. On withdrawal of the catheter to the right atrium a pressure gradient over the tricuspid valve of 19 mm Hg was found the mid diastolic pressure in the right atrium being 26 mm. The pressure tracings did not show the characteristic configuration of constriction of the heart. The cardiac index was 1.4 l per m and the arterial oxygen saturation 88.

At selective angiocardiology and cineangiography with contrast injection into the right atrium a mass was seen extending from the right atrium to the right ventricle impeding the flow through the tricuspid valve and through the outflow tract of the right ventricle (Fig 6). Intra-cardiac phonocardiography demonstrated a diastolic murmur in the inflow tract of the right ventricle.

At open heart operation in extracorporeal circulation a right atrial tumor of the size of a tennis ball was removed. The tumor had a short, broad stem to the atrial septum close to the foramen ovale and it extended towards the tricuspid valve which was normal. The tumor was soft and friable. The histological diagnosis was pseudomyxoma. The patient recovered slowly.

At follow up examination one year later the patient was asymptomatic with sinus rhythm, and the low voltage had disappeared.

SYMPTOMATOLOGY

Our review of the symptomatology is based on the case histories of 33 patients which have been published previously with sufficient information for our purpose and on our two patients presented above. The histological diagnosis was established at autopsy in three cases and at operation in the remaining 32 cases.

There were 15 males and 20 females. The age distribution at the time of the onset of symptoms is seen in Fig 7. The youngest patient was 7 months old and the oldest 57 years old.

The various symptoms and signs may be divided into four groups as seen in Table I.

Of the constantly present symptoms right heart failure was seen in all but two patients. It was obviously due to the mechanical obstruction caused by the tumor. Dyspnea was also commonly seen possibly due to a low cardiac output secondary to

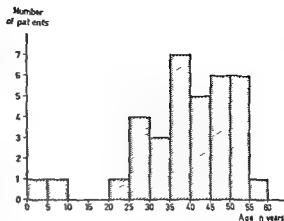


Fig 7 Age distribution of 35 patients with right atrial myxoma

the low venous return. The low cardiac output might also explain the cyanosis and the signs of insufficient peripheral circulation.

The intermittent symptoms which were seen in 22 patients were probably caused by a sudden increase in the obstruction to the blood flow through the tricuspid ostium e.g. provoked by the

Table I Frequency of symptoms and signs in 35 patients with right atrial myxoma

	No of pats	In total no of pats
Constant		
Right heart failure	33	0
Dyspnea during exercise	27	19
Dyspnea at rest	12	1
Cyanosis	8	7
Insufficient peripheral circulation	11	6
Leg cramps	2	
Gangrene	1	
Raynaud's symptoms	1	
Thrombophlebitis	2	
Intermittent		
Dyspnea	11	1
In certain positions	7	0
Nocturnal	2	0
Unprovoked	2	2
Cerebral symptoms	13	7
Syncope or dizziness	12	7
Epileptic seizures	1	0
Precordial or epigastric pain	13	0
Cyanosis	9	0
Flushing	4	0
General		
Fever	7	2
Weight loss	7	1
Pigmentation	3	0
Pulmonary embolism	6	1

Table II *Auscultatory findings in 35 patients with right atrial myxoma*

	No of pats
Diastolic murmur in tricuspid area	24
Systol & murmur in tricuspid area	21
Systolic/diastolic murmur only at the apex	2
Gallop rhythm	11
Friction rub	6
Normal findings	2
Total	35

Table III *Electrocardiographic findings in 35 patients with right atrial myxoma*

	No of pats
Right atrial hypertrophy	19
Right axis deviation	8
Low voltage	18
Right bundle branch block	11
Sinus rhythm	29
Atrial fibrillation	4
Intermittent atrio ventricular block	3
Entirely normal	2
Total	35

assumption of a new position as the left lateral recumbent position. The cerebral symptoms were often alarming and dominating. The precordial and epigastric pain was often intense but otherwise uncharacteristic.

Of the general symptoms which usually accompany tumors, fever and weight loss were each found in one fifth of the cases.

Pulmonary embolism occurred in six patients, the embolus consisting of either tumor tissue or thrombotic tissue. It was fatal in one case.

The duration of the symptoms until correct diagnosis averaged 3 years, ranging from five days to 36 years. The duration was less than one year in 25% of the cases.

Auscultation

As seen in Table II the auscultatory findings are mainly those of tricuspid valvular disease. The findings are often transient and may vary with the position of the patient.

The gallop rhythm was most likely due to tumor impaction as suggested by Sannerstedt et al. (26) and the friction rub was probably secondary to

affection of the pericardium as pericardial exudate was found in six cases.

The diastolic atrial murmur which was interrupted and synchronous with the P waves (Fig. 4) in our case 2 could easily be mistaken for a friction rub.

Electrocardiogram

The findings are summarized in Table III. In most cases a right-sided heart disease was indicated by signs of right atrial hypertrophy (peaked P waves in II, III, aVF or right-sided precordial leads), right axis deviation or right bundle branch block. As many as half of the patients had unexplained low voltage (Fig. 5).

Roentgenogram of the chest

A roentgenogram of the chest was obtained in all patients but one. In 22 cases cardiomegaly was noted. A prominent right heart border was seen in 18 cases. In five patients the heart was normal.

In four patients calcifications within the cardiac silhouette were noticed. Pleural exudate was demonstrated in four cases. Pulmonary congestion was not noted in any case.

Laboratory tests

These were uncharacteristic in some cases, consisting of anemia, leucocytosis and elevated sedimentation rate.

Right heart catheterization

Heart catheterization was performed in 27 patients. In all but three patients a diastolic pressure gradient was measured across the tricuspid valve. In two patients a pressure gradient was found over the pulmonary valve and over the outflow tract of the right ventricle, respectively. One patient had pulmonary hypertension due to recurrent tumor embolism.

Cardiac output was measured at rest in 13 patients. Seven patients had either a cardiac output lower than 3.5 l per min or a cardiac index lower than 2 l per square meter per min.

Angiocardiography

Selective angiocardiography with contrast injection into the right atrium was performed in 25 cases without complications. In all cases the tumor was demonstrated.

Table IV Initial clinical diagnosis of 35 patients with right atrial myxoma

	No of pats
Pericarditis	8
Unspecific chronic	5
Tuberculous chronic	2
Acute	1
Mitral stenosis	4
Tricuspid stenosis	4
Rheumatic fever	2
Ebstein's disease	2
Myocardial infarction	2
Carcinoid tumor	2
Myocarditis	1
Bacterial endocarditis (subacute)	1
Peripheral arterial insufficiency lower extremities	1
Polycythemia vera	1
Addison's disease	1
Rheumatoid disease (arthritis)	1
Functional symptoms	1
No information	4
Total	35

Prognosis and treatment

Three patients died without attempts at surgery the cause being heart failure in two cases and pulmonary embolism in one case. In the remaining 32 cases surgery was performed.

Eight cases were operated on without extracorporeal circulation and four died. Twenty-four cases were operated on in extracorporeal circulation with 16 survivors. The usual finding at operation was a pedunculated tumor by which the intermittent symptoms could be explained. Tricuspid insufficiency following operation was reported in five cases.

DISCUSSION

The clinical diagnosis may be difficult as seen from the listing of the initial diagnosis in the 35 patients in Table IV.

As soon as a myxoma has reached a certain size it will give rise to symptoms of which right heart failure is dominant. In the presented material the lack of pulmonary congestion on the roentgenogram and the majority of the electrocardiographic findings clearly pointed to the right side of the heart as site of the disease.

Isolated right heart failure is found in pulmonary diseases, pulmonary stenosis, disease of the right ventricle, constrictive pericarditis, tricuspid valve disease and tumor of the right atrium. The first

possibility is easily ruled out. Auscultation will in the majority of cases reveal a tricuspid murmur which in combination with a history of intermittent cardiac or cerebral symptoms should arouse the suspicion of myxoma. Non valvular calcifications inside the cardiac silhouette point to the same possibility.

Furthermore right heart catheterization which would be indicated in cases with isolated right heart failure will usually show an isolated tricuspid stenosis and exclude constrictive pericarditis, pulmonary valve disease and disease of the right ventricle.

As isolated tricuspid stenosis is extremely rare, angiocardigraphy should be performed in all cases of apparent isolated tricuspid stenosis as well as in cases of isolated right heart failure in which the heart catheterization fails to give a definite diagnosis.

Selective right atrial angiocardigraphy will demonstrate the tumor. No complication to this examination in cases with right atrial myxomas has been reported.

Removal of the tumor by open heart surgery must be performed.

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THE ADRENOCORTICOTROPIC EFFECT
OF β -SERINE¹ NORLEUCINE⁴ VALINAMIDE³ β 1 25 CORTICOTROPIN
(DW 75)

A Comparison with Genuine ACTH

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Abstract A comparison has been made in 15 healthy individuals between the adrenocorticotrophic effect of a synthetic pentacosapeptide (DW 75) and natural ACTH. The synthetic preparation was found to be six times more potent than the genuine preparation and to have a slightly longer lasting effect.

Natural adrenocorticotrophic hormone obtained from various species consists of a single unbranched sequence of 39 amino acid residues. Species variations occur but are confined to the amino acids that occupy positions 25 through 33. The active portion of the molecule (positions 1 through 24) is identical in all corticotropins investigated. Thus only a portion of the ACTH polypeptide chain starting from the amino-terminal end is necessary for the full biological potency of ACTH.

In 1961 Kappeler and Schwyzer (7) synthesized a tetracosapeptide containing 24 amino acids corresponding to the first 24 amino acids of the genuine ACTH. This preparation has been extensively studied and found to have a biological effect identical with the natural hormone. The activity of the tetracosapeptide equals 106 IU/mg whereas the activity of the natural corticotropin equals 15 IU/mg.

In 1966 Boissonas et al (1) succeeded in synthesizing a pentacosapeptide more resistant than the genuine hormone and its active synthetic analogue so far prepared. It contains D-serine at the terminal and valinamide at the carboxyl terminal. The methionine residue in position 4 is replaced by an isologous norleucine residue. The analogue is a D-serine¹ norleucine⁴ valinamide-³ β 1 25-corticotropin and has been named DW 75. This pentacosapeptide was shown by Doepfner

(4) to have an activity of 625 IU/mg thus being six times more potent than genuine ACTH and its 1-24 analogue. In man DW 75 has been found very active. But as only preliminary studies have been made to compare the adrenocorticotrophic effect of DW 75 and genuine ACTH a study of the effect in a larger series of healthy individuals was performed.

MATERIAL AND METHODS

Fifteen healthy individuals aged 20-58 years were examined. They were doctors, nurses and laboratory technicians working at the University Clinic Rikshospitalet, Oslo. In order to eliminate the endogenous secretion of ACTH, all individuals were given dexamethasone orally the first five individuals 0.5 mg, the others 2 mg 8 h before the test and at the time of the DW 75 or ACTH injection. DW 75 (supplied by Sandoz Ltd, Basel Switzerland) and ACTH (Corticotropin, Nycor) were given slowly (1 min) by intravenous injection. Blood was taken for plasma 17-hydroxycorticosteroid (17 OHCS) estimation in heparinized tubes immediately before the injection and after 1, 2, 4 and 8 h. The blood samples were spun in the centrifuge for separation of plasma and blood cells and the plasma was frozen. All plasmas from individual tests were analyzed simultaneously.

Every individual was examined four times, being given 2 injections of 5 and 10 IU of DW 75 and 5 and 10 IU of ACTH. The interval between the tests in the same individual was at least four days.

Plasma 17 OHCS concentration was determined by a modification of the Elk Nes (5) method as described by Bruck-Johnsen and Solem ().

No side effects were observed in any individual. In four individuals redness of the face was observed on the day following the test day, probably due to an ACTH effect. In this respect there was no difference between DW 75 and genuine ACTH.

Table I Mean increment of plasma 17 OHCS concentration ($\mu\text{g}/100\text{ ml}$) in 15 dexamethasone blocked healthy individuals 1 2 4 and 6 hours after i.v. injection of DW 75 and ACTH respectively

	1 h	2 h	4 h	6 h
DW 75 5 IU	12.6	15.6	10.5	2.6
ACTH 5 IU	15.3	12.8	6.7	0.8
DW 75 10 IU	11.6	16.3	10.3	2.7
ACTH 10 IU	14.0	12.8	6.7	0.5
P	<0.10	<0.05	<0.01	0.05

Pooled values of DW 75 and ACTH respectively

RESULTS

Oral administration of dexamethasone 8 h before the injection of DW 75 or ACTH suppressed the endogenous production of cortisol satisfactorily. At 0 h the concentration of plasma 17 OHCS for all tests averaged $1.7\text{ }\mu\text{g}/100\text{ ml}$. The increment of plasma 17 OHCS concentration above the value stated at 0 h is considered due to the effect of DW 75 or ACTH. The changes in plasma 17 OHCS concentrations are shown in Table I.

After 5 IU of DW 75 the plasma 17 OHCS concentration increased by $12.6\text{ }\mu\text{g}/100\text{ ml}$ at 1 h, $15.6\text{ }\mu\text{g}/100\text{ ml}$ at 2 h. At 4 h the increment was $10.5\text{ }\mu\text{g}/100\text{ ml}$ and at 6 h $2.6\text{ }\mu\text{g}/100\text{ ml}$.

An almost identical response was obtained for 10 IU of DW 75. At 1 h the plasma 17 OHCS was $11.6\text{ }\mu\text{g}/100\text{ ml}$ at 2 h $16.3\text{ }\mu\text{g}/100\text{ ml}$ thereafter 10.3 and $2.7\text{ }\mu\text{g}/100\text{ ml}$.

Five and ten IU of ACTH increased the plasma 17 OHCS concentrations in the same degree the levels being 15.3 and $14.0\text{ }\mu\text{g}/100\text{ ml}$ respectively. Later on the values decreased slowly being almost identical with the 0 value at 6 h.

Since identical effects were found for both dosages the results have been pooled for statistical evaluation. Statistically the values obtained for DW 75 are higher than those for genuine ACTH 2 4 and 6 h after injection ($p < 0.05 < 0.01 < 0.05$ respectively) while there is no difference between the effects of the two preparations at 1 h. Also there is no difference between the maximal increments above 0 h for the two preparations.

DISCUSSION

In this study two important observations were made namely regarding the potency of the penta-

cosapeptide and the duration of its effect. The maximal response of the two preparations was identical and since the activity of DW 75 equals $625\text{ IU}/\text{mg}$ or $10\text{ IU}/0.016\text{ mg}$ whereas 10 IU of genuine ACTH equals 0.1 mg it has been shown that DW 75 is six times more potent than genuine ACTH and its β^{1-4} analogue. This is a confirmation of Doepfner's (4) study using the ascorbic acid depletion assay *s.c.*

Apparently DW 75 also has a longer lasting effect than genuine ACTH: the plasma 17 OHCS values being higher for DW 75 than for the genuine preparation both at 2 4 and 6 h. At 6 h after the injection of DW 75 the plasma 17 OHCS level is still $2.7\text{ }\mu\text{g}/100\text{ ml}$ whereas the plasma 17 OHCS level after ACTH has fallen to a very low level.

This shows a more prolonged effect of DW 75 confirming the findings of Jenny *et al.* (6).

This study gives no indication of the reason for the stronger and longer lasting effect of DW 75. It is well known that intravenously injected ACTH will disappear rapidly from the blood and blood disappearance half time value has been found to be only a few minutes. According to Meakin and Nelson (8) this is due to a rapid enzymatic inactivation which can be prevented by heating the plasma to 60°C or adding cysteine. However it also has been maintained (3) that intravenously injected ACTH is concentrated in the kidney and the liver and from these organs is given off little by little to act on the adrenals.

The chemical configuration of DW 75 gives no support to either of these possibilities but a retardation of the enzymatic degradation is most likely. The substitution of D-serine for L-serine makes this preparation more resistant to degradation by aminopeptidases whereas valinamide in stead of asparagine in position 25 increases the resistance to degradation by carboxypeptidases. Furthermore it is well known that inactivation of the molecule results from the oxidation of methionine to methioninesulphoxide. However in DW 75 this is prevented by substituting methionine by its isologue norleucine. In this way a very potent preparation has been synthesized possessing greater stability in the plasma and the tissues.

Most likely DW 75 possesses also another advantage as a synthetic polypeptide: it has no tendency to cause allergic reactions. No such reactions were observed in this study.

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Table I Muscle glycogen in chronic renal failure

Case	Sex	Age	Diagnosis	Diet ^b	Plasma		Glycogen	
					Creatinine (mg/100 ml)	Urea N (mg/100 ml)	(g/100 g wet weight)	(g/100 g glycogen and fat free solids)
G B	♀	38	CP	PR	14.6	118	0.83	4.04
K L	♀	31	CG		14.3	150	1.19	6.31
B L	♀	36	CP	IR	14.0	174	1.46	7.28
D A	♀	67	CP	PR	17.3	130	1.05	5.42
K F	♀	60	CP	PR	2.4	160	2.08	11.4
T L	♂	61	CP	PR	10.0	120	1.16	5.82
M S	♂	40	NS	PR	11.7	90	1.22	6.31
B D	♂	32	NC	PR	13.2	97	1.30	6.13
A T	♂	48	PC		16.4	147	1.58	7.91
A J	♂	53	PK		12.5	100	1.14	5.93
B B	♂	51	PK	PR	21.0	190	1.20	6.12
A H	♂	46	NS		10.2	85	1.34	6.89
L A	♂	18	CG	PR	15.2	105	2.14	11.2
B S	♂	18	CG		10.7	101	1.76	8.59
L L	♂	20	PC		16.0	97	1.42	6.86
B G	♂	17	CG	PR	16.2	140	1.27	6.88
S T	♂	45	PK		11.4	144	0.83	4.46
B S	♂	50	PC	PR	18.1	111	1.70	6.29
P M	♂	59	CG		18.8		1.25	6.00
R H	♂	43	NS		15.8		1.28	6.56
J S	♂	36	CG		25.2		1.18	5.56

CP = chronic pyelonephritis CG = chronic glomerulonephritis NS = nephrosclerosis PK = polycystic kidney disease
^b PR = protein restriction

Acute renal failure (12 patients) See Table II

This group was heterogeneous with respect both to etiology and duration of renal failure. In seven cases the acute renal failure was a complication of surgery. Although during or after operation all the patients had a fall in blood pressure and other signs of shock, all except K. W. had normal or raised blood pressure at the time of investigation. Prior thereto case S. N. had undergone peritoneal dialysis for two days and had in addition received a large amount of 30% glucose i.v.

Of the other patients two had had a fall in blood pressure in connexion with the acute onset (S. F. and B. L.) but had normal blood pressure when the examination was made. After five days anuria case S. F. had undergone peritoneal dialysis for two days and case B. L. had been started two days before.

Methods

Muscle tissue was obtained from the quadriceps femoris muscle by means of needle biopsy (3). The trichloroacetic acid extractable glycogen was determined in duplicate muscle specimens according to Hultman (15). The neutral fat and water content were determined in separate muscle samples. Since many of the uremic patients had an increased water content of the muscle and several also an increased content of neutral fat, the glycogen values were referred to glycogen and fat free solids as well as to wet weight.

RESULTS

1 Chronic renal failure (see Table I)

All patients had normal muscle glycogen values with the exception of cases G. H. and S. T. who had slightly decreased values. In a comparison with both normal healthy subjects and with hospitalized patients without renal failure or diabetes mellitus no significant difference was found between the mean values (Table III).

2 Acute renal failure (see Table II)

All but two patients had decreased muscle glycogen values. Particularly in the group of post-operative renal failure most patients had extremely low values. The only patients in this group who had normal values (S. N. and S. F.) had undergone peritoneal dialysis and hemodialysis respectively a short time before. Case B. L. is of special interest. When the first biopsy was performed diuresis had started and her serum creatinine had started to fall. Her muscle glycogen value was extremely low. During the following weeks she ate a normal hospital diet without

Table II Muscle glycogen in acute renal failure

Case	Sex	Age	Diagnosis	Days of oliguria diuresis	Plasma		Glycogen	
					Creatinine (mg/100 ml)	Urea N (mg/100 ml)	(g/100 g wet weight)	(g/100 g glycogen and fat free solids)
<i>A Postoperative renal failure</i>								
T J	♂	44	Op aortic stenosis and incompetence	3	15.4		0.51	2.59
S B	♂	44	Op aortic stenosis	5	18.0		0.31	1.51
S N	♀	54	Op ileus	9	9.0		1.12	5.54
K A	♂	34	Cholecystectomy	9	15.6		0.51	2.24
K W	♀	70	Partial gastrectomy pancreatitis and peritonitis	8	10.7		0.14	0.67
E T	♂	39	Partial gastrectomy	5	17.2		0.38	1.44
H B	♂	57	Cholecystectomy and partial gastrectomy	4	13.6		0.70	3.75
<i>B Other cases</i>								
A V	♀	72	Hemolytic crisis	8	9.8		0.72	
V L	♀	61	Fever hemolytic crisis	9	9.8		0.81	3.89
S F	♂	32	Carbon tetrachloride poisoning	9	22.4		1.15	
A S	♀	37	Toxemia of pregnancy liver affection	1	4.2	74	0.55	3.42
B L	♀	59	Gastroenteritis endotoxin shock	3	6.8		0.27	1.37
				7	1.1		(0.66)	(3.34)
				17	1.2		(2.38)	(13.37)

restriction of protein or calories. One week later the muscle glycogen had not been normalized. When a determination was made after a further 10 days the muscle glycogen value was high.

Table III Muscle glycogen in control subjects and in patients with renal failure

	n	Glycogen (g/100 g wet weight)	Mean and range (g 100 g glycogen and fat free sol ds)
subjects	28	1.39 (0.9-2.49)	6.69 (4.33-12.66)
hospitalized non-uremic and non-diabetic patients	16	1.40 (1.01-2.12)	
chronic renal failure	21	1.31 (0.83-0.8)	6.93 (4.04-12.2)
Acute renal failure			
A Postoperative	7	0.51 (0.14-1.12)	2.53 (0.67-5.5)
B Other cases	5	0.70 (0.27-1.15)	2.89 (n=3) (1.35-3.74)

DISCUSSION

1 Chronic renal failure

It is known that in man the basal metabolism of the skeletal muscle is maintained chiefly by fat combustion (2) whereas the muscle carbohydrate store is mainly utilized in muscle work (6, 16). In diabetes mellitus there is no decrease in the ability to utilize muscle glycogen in muscle work (personal unpublished observations) but on the other hand the resynthesis of muscle glycogen is impaired due to insulin deficiency or a decreased sensitivity to insulin. In juvenile diabetics we previously found (7) greatly reduced muscle glycogen values (mean 0.49 g/100 g wet weight). Adults with mild diabetes not requiring insulin also had significantly lower values (mean 1.11 range 0.77-1.56 g/100 g) than non-diabetic hospitalized patients and normal subjects (28).

Some of the patients in this study who were on a low protein diet presumably had a relatively high carbohydrate intake. On the other hand several of them were greatly affected clinically by uremia with anorexia and vomiting during the

period before the glycogen determination. The effect of both fasting and a high-carbohydrate diet on the muscle glycogen in normal experimental subjects indicates that these diets have only an inappreciable effect unless heavy muscular work is performed before and during the examination (17). It is actually noteworthy that practically all these severely ill patients had completely normal muscle glycogen values.

The fact that we found entirely normal muscle glycogen values in so many chronically uremic patients argues against the decreased sensitivity to insulin described in uremia involving the skeletal muscle to any great degree. Campanacci et al. (9) recently reported low muscle glycogen values in patients with chronic uremia which they interpreted as an expression of the same disturbance as causes decreased glucose tolerance. Their results are not in agreement with those presented here. Important differences however exist between the two series. Thus most of the patients of Campanacci et al. had been on extremely protein poor diet for a varying time before the determinations. This may have contributed to the reduced glycogen values since lengthy protein deficiency is known to be able to affect the carbohydrate metabolism in man (22). A decreased glucose tolerance also in dogs given a low protein diet (13). Moreover it is possible that the biopsy specimens of Campanacci et al. had a relatively high content of connective tissue in relation to muscle cells since in many cases they noted extremely low values for alkali soluble protein nitrogen in relation to fat free dry weight.

2. Acute renal failure

The patients with acute uremia form a much more heterogeneous group than those with chronic uremia. In all but two patients who had undergone dialysis the muscle glycogen content was low. Low values were recorded particularly in patients whose renal failure had developed as a complication of operative trauma.

It is known that the glycogen content of the skeletal muscle falls considerably after an abdominal operation (5). We have also observed that on the fourth to fifth day after operation the patients have a decreased synthesis of glycogen after a standardized glucose infusion as compared to normal experimental subjects (unpublished observations). The postoperative stage has been de-

scribed as a pseudo-diabetic state (23). In addition shock is known to lead to a rapid breakdown of muscle glycogen in experimental animals (20). The extremely low muscle glycogen values which we found in postoperative patients with renal failure may therefore have arisen as a sequelae of the operative trauma with resulting shock, i.e. the same factors as led to the patients' renal damage. No definite conclusions can be drawn from the type of case material with acute anuria in our study about whether the acute uremic intoxication as such had any effect on the muscle glycogen or whether the reduced muscle glycogen values constitute an unspecific component of these patients' catabolic state.

It is known that potassium and glycogen accumulate together in liver and muscle (4, 11) approximately in proportion 0.5 mEq K per g glycogen. A rapid breakdown of muscle glycogen, as occurs in acute renal failure, will liberate considerable amounts of potassium from the cells and thus contribute to the development of hypokalaemia.

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PERMANENT ECTOPIC SUPRAVENTRICULAR TACHYCARDIA TREATED WITH AN ADRENERGIC β RECEPTOR ANTAGONIST

A Case Report

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Abstract A case of permanent ectopic supraventricular tachycardia followed for 11 years, the last two years on propranolol treatment is reported. Untreated the heart rate varied spontaneously between 140 and 210 per min. Quinidine, procainamide, Valsalva's maneuver and carotid massage had no effect. Acetylcholine intravenously and electroshock converted to sinus rhythm which however persisted only for a few seconds. Digitalis treatment had no effect on the atrial rate but caused a varying degree of A-V block (grade II-III). When the patient was treated with propranolol the ectopic focus was retarded until the sinus node could compete. However when the treatment was discontinued there was a gradual acceleration until the firing of the ectopic focus which again became the leading pacemaker. This indicates that the sinus node was less sensitive to adrenergic β receptor blockade than the ectopic focus. This fact and the chronicity of the tachycardia are in accordance with a supposed hypersensitivity of the ectopic focus to sympathetic stimulation.

A case of long lasting ectopic supraventricular tachycardia was first reported by Wilson and Herrman (4) in 1923. In 1954 Shachnow et al (3) were able to find only 13 reported cases of more than 2 months duration. Since then another eight cases, mostly children, have been added to literature (1).

In the following a case of such long lasting atrial tachycardia is reported in which with an adrenergic β receptor antagonist (Inderal®) suppressed the tachycardia.

CASE REPORT

The patient, a 30-year-old woman was first admitted to Ullevaal Hospital Department VIII in 1957 because of thyrotoxicosis and tachycardia. She had never been hospitalized before. In her family no case of heart disease was known.

In the patient's childhood her mother had observed rapid huge pulsations in the carotids and shortness of breath on slight exertion, but a doctor was never consulted. The patient felt relatively well however until the age of 30 when, during an attack of gastroenteritis, she became weak and dyspnoeic. The doctor called for found a tachycardia and gave her digitalis. As she did not improve she was referred to our hospital.

Physical examination (16/9/1957) revealed a somewhat nervous woman but otherwise with a healthy appearance. The heart rate was 150 per min and the blood pressure 115/85 mm Hg. The heart sounds were normal without murmurs and the relative heart volume was also normal (400 ml per square meter body surface). She had no signs of hyperthyroidism and the thyroid function tests were normal. ECG from the digitalized patient (Fig. 1) showed a supraventricular tachycardia with partial A-V block (grade II-III). Quinidine, procainamide, Valsalva's maneuver and carotid massage did not convert the arrhythmia. The patient was given 0.10 m^g acetylcholine intravenously and a slower rhythm (rate 88 per min) with normal P waves (probably sinus rhythm) was seen but only for a few seconds. The patient was discharged with digoxin 0.10 mg per day and she still had an atrial rate varying between 140 and 200 per min but with A-V block grade II and a ventricular rate varying between 100 and 150 per min. After some weeks of rest she was able to return to her office work. In the following years the situation was unchanged. In 1965 she complained of some dyspnoea, but she had no signs of congestive heart failure. In 1966 she felt tired, dyspnoeic and could barely manage light office work but a re-investigation did not reveal any signs of heart disease except the atrial tachycardia. Electrocardiogram was now tried but after a few sinus beats the atrial tachycardia continued.

The digitalized patient was then given propranolol (Inderal®) 10 mg intravenously (Fig. 2) and the atrial rate was reduced from 160 to 116 per min. The slower atrial rate led to a 1:1 A-V conduction with prolonged PQ interval and the ventricular rate therefore increased from 100 to 116 per min. All treatment was then stopped for a month without obvious clinical deterioration of the

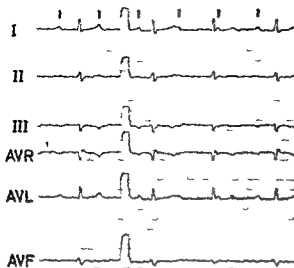


Fig 1 ECG 179 1957 of the digitalized patient. A supra ventricular tachycardia with varying degree of A V block is seen. The atrial waves (rate 140 per min) are marked. Ventricular rate 95 per min. Paper speed 50 mm per sec.

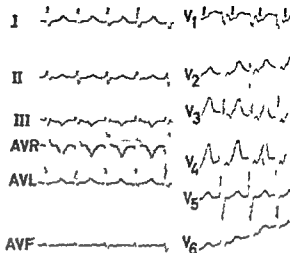
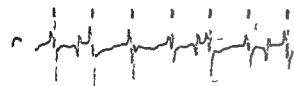


Fig 3 ECG 162.1966 of the resting patient after one month without drug therapy. The original tachycardia (rate 190 per min) with 1:1 conduction is seen. The atrial waves are marked in lead V. Paper speed 40 mm per sec. Lead aVR 20 mm = 1 mV.

A=162 V=100 DIGITALIS



A=V=126 DIG + PROPRANOLOL

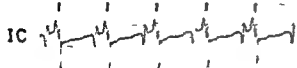


Fig 2 ECG 101 1966 of the digitalized patient showing the supraventricular tachycardia before (upper) and after (lower) 10 mg propranolol intravenously. After propranolol atrial retardation occurred but A V conduction improved to 1:1 and the ventricular rate therefore increased. IC = intracardiac ECG recorded from an electrode in the right atrium. The intracardiac atrial waves are marked. I = first standard lead. Paper speed 50 mm per sec.

patient. The ECG now showed a constant 1:1 A-V conduction (Fig 3). The ectopic focus seemed to be located to the lower anterior portion of the right atrium, the atrial depolarization going dorsolaterally towards the left. The rate of firing of the ectopic focus changed spontaneously from 140 per min during sleep to 70 per min after slight exercise or with the patient standing. Propranolol 90 mg per day orally which was now given, reduced the atrial and ventricular rate to 80 per min and some normal P waves appeared (Fig 4). Propranolol 120 mg per day stabilized the sinus rhythm at the resting rate of 60 per min (Fig 4). On exercise some supraventricular

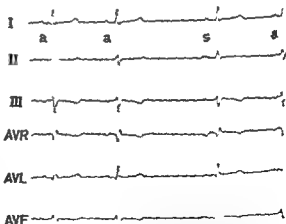


Fig 4 ECG 18 1966 during treatment with 90 mg propranolol per day. A retarded ectopic atrial focus (a) rate 80 per min and one sinus beat (s) are seen. Paper speed 50 mm per sec.

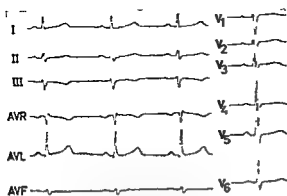


Fig 5 ECG 7.0.3.1966 during treatment with propranolol 1.0 mg per day. Regular sinus rhythm with normal P waves is seen. Lead aVL and V_4 20 mm = 1 mV. Paper speed 50 mm per sec.

beats still occurred, but the heart rate remained within normal limits. From then on the patient has taken propranolol 1.0 mg per day and sinus rhythm has persisted. During the first week on propranolol she felt a bit drowsy and nervous because she "could not feel the heart beat in her chest". Later when she noticed the slower and regular pulse and her dyspnea improved she started training in gymnastics and swimming.

In November 1966 the propranolol treatment was stopped for 36 hours. The ectopic atrial focus soon accelerated to 160 per min, the normal P waves disappeared and the patient's dyspnea and fatigue returned. When propranolol treatment was started again, the normal heart rate was reestablished, as was her sense of well-being. Up to date (December 1968) she still feels well and has no complaints.

DISCUSSION

Chronic supraventricular tachycardia may be divided into a sustained and a repetitive type (2, 3). No certain difference in the long-term prognosis between the two types has been documented (2).

In our patient the tachycardia is of the sustained type. It probably started in childhood but was first recorded at the age of 30. She has been under treatment since 1957 and until propranolol treatment was started in 1966 ectopic atrial tachycardia was constantly registered. Acetylcholine intravenously (in 1957) and electroshock (in 1966) had a transient conversion to sinus rhythm but it did not persist for more than a few seconds. Propranolol however suppressed the ectopic focus and since March 1966 she has taken 120 mg per day. At every examination a regular sinus rhythm of about 60 per min has been registered. However

as soon as propranolol was discontinued the ectopic center immediately starts the tachycardia anew.

In most of the reported cases the sustained ectopic supraventricular tachycardia has been susceptible to a variety of stimuli and procedures in which the sympathetic and parasympathetic tonus seems to play a part. It was therefore not surprising that the tachycardia was retarded by adrenergic β receptor blockade but unlike the β receptor blockade in ordinary paroxysmic atrial tachycardia in which a conversion to sinus rhythm usually takes place the adrenergic β receptor blocking drug in this case of chronic supraventricular tachycardia caused a retardation of the ectopic focus and a competition between the sinus node and the ectopic focus occurred. When 90 mg per day of propranolol was given the retarded ectopic focus was the leading pacemaker and the sinus node just captured a few beats. When 120 mg per day was given the sinus node was the main pacemaker but the ectopic focus could still capture a single beat now and again. This means that the tachycardia was never converted to sinus rhythm; the ectopic focus was only retarded to a rate where the sinus node could compete, indicating that the sinus node in this case is less sensitive to adrenergic β receptor blockade than the ectopic atrial focus. This fact and the chronicity of the tachycardia are in accordance with a supposed hypersensitivity of the ectopic focus to sympathetic stimulation.

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HEMODYNAMIC EFFECTS OF PROPRANOLOL (INDERAL®) AND H 56/28 (APTIN®)

IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

A Comparative Study

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Abstract The hemodynamic effects of propranolol (Inderal®) and H 56/28 (Aptin®) were compared in a study of 17 patients in the acute stage of myocardial infarction. Eight patients received 5 mg propranolol i.v. (group I) and the remainder 5 mg H 56/28 (group A). Cardiac index (CI), heart rate (HR) and stroke index (SI) were significantly reduced after both drugs. The difference between mean CI and SI reductions in the two groups was not significant ($p > 0.05$) while the difference between mean HR reductions was almost significant ($0.05 < p < 0.05$). Total peripheral resistance index (TPRI), circulation time (CT) and right atrial mean blood pressure (RAMP) increased significantly in both groups. The difference between mean TPRI, CT and RAMP increases in the two groups was not significant for TPRI ($p > 0.05$) but a most significant for CT and RAMP ($0.01 < p < 0.05$). Arterial systolic blood pressure (SBP) fell almost significantly ($0.05 < p < 0.05$) in group I while no change occurred in group A. Arterial diastolic and mean blood pressures did not change in either group.

It is concluded that propranolol and H 56/28 have about equal effects on most of the measured parameters in patients with strong sympathetic drive. The intrinsic sympathetic stimulating action of H 56/28 is not sufficient to compensate for the β adrenergic receptor blocking effect of the drug in this situation. The two drugs should therefore both be considered dangerous in patients in whom augmented sympathetic drive is necessary for the maintenance of adequate cardiac contractility and rate. A tendency to bradycardia, prolonged circulation time and hypotension may be slightly less after H 56/28 than after propranolol injected intravenously in the same doses.

β adrenergic receptor blocking drugs have proved their usefulness in the treatment of certain cardiac arrhythmias (10, 13, 14, 16, 18, 19) in pectoris (1, 4, 6, 7, 11) and in some other conditions in which normal or augmented adrenergic tone plays a part in the pathogenesis. For a review see Epstein and Braunwald (2).

The therapeutic and hemodynamic effects of

these drugs mainly depend on their β adrenergic receptor blocking properties but their non specific myocardial depressive (9, 21) and non specific anti arrhythmic properties (15, 20) may also be of importance.

The β adrenergic receptor blocking drugs in present use are either almost solely β adrenergic receptor antagonists or β adrenergic receptor antagonists with some intrinsic β sympathomimetic properties. It still seems uncertain however whether the latter kind of drug in equipotent doses is less dangerous than the former in patients with impending heart failure in whom sufficient myocardial contractility and heart rate are dependent on augmented adrenergic tone.

When investigating the hemodynamic effects of new β adrenergic blocking drugs it seems important to test the drugs also in patients with relatively high sympathetic tone. This probably occurs in the acute stage of myocardial infarction or during physical exercise. On the contrary in resting recumbent persons with no known cardiac affection the adrenergic activity is presumably small (12). Thus the hemodynamic consequences of β adrenergic receptor blockade under these conditions would be limited provided that the agent does not exert sympathomimetic action.

In the present study of patients in the acute stage of myocardial infarction an attempt was made to compare the hemodynamic effects of propranolol (Inderal®) which is a quite specific β adrenergic receptor antagonist with those of H 56/28 (Aptin®) which besides being a potent β adrenergic receptor blocking drug also has some β sympathomimetic properties.

by propranolol than by H 56/28. The systolic blood pressure fell almost significantly after propranolol, while no change was seen after H 56/28.

Propranolol and H 56/28 both have important and about equal effects on most of the measured parameters in patients with strong sympathetic drive. The intrinsic sympathetic stimulating action of H 56/28 is not sufficient to compensate for the β adrenergic receptor blocking effect of the drug in this situation. The two drugs should therefore both be considered dangerous in patients in whom augmented sympathetic drive is necessary for the maintenance of adequate cardiac contractility and rate. The tendency to bradycardia prolonged circulation time, rise of right atrial pressure and hypotension may be slightly less after H 56/28 than after propranolol injected intravenously in the same doses.

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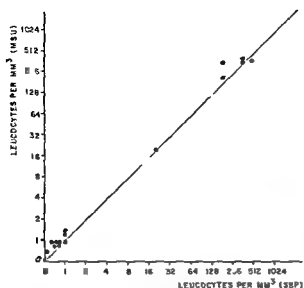


Fig 1 Comparison of leucocyte counts in mid-stream urine (MSU) and in urine aspirated by suprapubic bladder puncture (SBP)

cover slip (4) only clearly visible cells were counted (depth of the field approximately 0.025 mm)

Renal concentration ability and the leucocyte excretion rate have been estimated simultaneously. At 4 p.m. 5 IU of Pitressin[®] Tannate in Oil (Parke Davis & Co) were given subcutaneously (2). The exact time of the first voiding on the next morning was noted. During the next day a midstream specimen was collected. Estimation of diuresis per hour was based on the total volume of urine of the second voiding and the time interval between the first and the second voidings. A specimen from each of these voidings was stored at -18°C for later determination of the osmolality.

The mid stream specimen from the second voiding was used for another urine culture and leucocyte count. After careful stirring of the specimen, one drop of Sternheimer Malbin's or Prescott's stain was added to one ml of the urine. Leucocytes were counted in a Fuchs-Rosenthal counting chamber. Two hundred, or all leucocytes in 3.2 mm² were counted (5).

Disrupted or degenerated cells were disregarded. The coefficient of variation in cell counting has been found to increase from 9 to 17 as the number of leucocytes decreases from 65 to 4 per mm². The counting results were independent of the staining method employed.

The osmolality of the urine specimens was estimated at Medical Department II Rigshospitalet, Copenhagen, by determination of the freezing point depression with an Advanced Osmometer.

RESULTS

In 25 cases the leucocytes in urine aspirated by suprapubic puncture and in mid stream specimens collected in connection with the puncture were

counted. Fig 1 shows good agreement between the leucocyte concentration in the bladder urine and in mid stream specimens.

The number of leucocytes per mm³ of urine has been compared with the leucocyte excretion per hour. The urine from 97% of 164 patients who excreted more than 400 000 leucocytes per hour contained more than ten leucocytes per mm³. Ten or fewer leucocytes per mm³ were found in 93% of 71 cases excreting fewer than 400 000 per hour.

With the technique employed for microscopic examination of urinary sediment it has been demonstrated that all patients with three or more leucocytes per HPF excreted more than 400 000 leucocytes per hour (19). If there were fewer leucocytes per HPF pyuria could not however be ruled out.

The results of microscopic examination of the urinary sediment of all patients referred to the clinic are shown in Table I. Even though three or more leucocytes were found in 75% of the 219 patients with bacteriuria and in 38% of the 253 women without significant bacteriuria, the probability of bacteriuria increased with the number of leucocytes seen per HPF.

In 144 women of 152 with bacteriuria the leucocyte excretion rate exceeded 400 000 per hour (Fig 2). The excretion rate was found to be of the same magnitude in bacteriuric patients regardless of the type of organism isolated from the urine. None of the 42 convalescents with no history or sign of renal disease excreted more than 400 000 leucocytes per hour.

Table I Maximum number of leucocytes per HPF seen by microscopic examination of the sediment in urine specimens from 472 women with symptoms of urinary tract infection

	Leucocytes per HPF				Unknown	Total
	0-2	3-5	6-20	>20		
No. of patients without bacteriuria	158	32	37	22	4	253
No. of patients with bacteriuria	54	31	85	63	1	19
All patients examined	212	63	102	90	5	472
Per cent with bacteriuria	25	49	64	76		

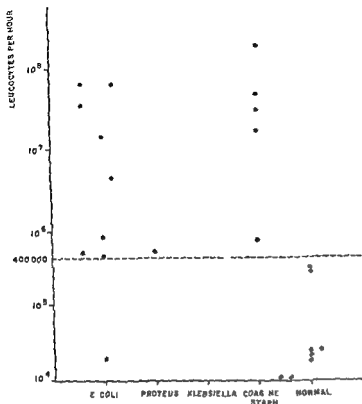


Fig 2 Leucocyte excretion rate per hour in 42 normal subjects and in 15 women with urinary tract infection shown in relation to the infecting organisms

Since February 1967 the maximal urine concentration ability (2) has been investigated in the bacteriuric patients. In these patients impaired concentration ability is regarded as a sign of pyelonephritis (13-32). In 129 patients the leucocyte excretion as well as the concentration ability was determined. It was found that the leucocyte excretion tends to increase as the concentration ability decreases (Fig 3). All but one of 26 patients who excreted fewer than 10^6 leucocytes per hour had a concentration ability of more than 750 mOsm/kg which is considered normal (2).

In four patients with uncomplicated urinary tract infection the leucocyte excretion rate was studied during treatment. On the second day of treatment all the patients had sterile urine and the leucocyte excretion rate decreased rapidly within the first few days (Fig. 4).

All the patients with bacteriuria were placed under continued observation. In 46 cases of reinfection (44 patients) the last urine specimen examined before recurrence of bacteriuria was ab-

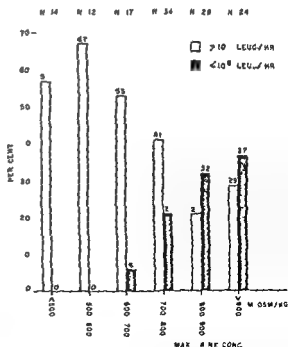


Fig 3 Leucocyte excretion in relation to the maximal urine concentration ability in 179 women with bacteriuria.

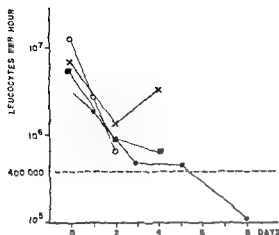


Fig. 4 Leucocyte excretion rate during the first days after start of treatment

tained after a period of at least two months with sterile urine. Table II shows the results of microscopic examination of urinary sediment before and during the period of bacteriuria and the first weeks after the start of treatment. Three or more leucocytes per HPF were found in 15% of the specimens examined before reinfection and in 78% of the specimens collected during the period of bacteriuria. After one month with sterile urine three or more leucocytes per HPF were seen in 13% of the specimens.

DISCUSSION

The diagnosis of pyuria

Simple microscopic examination of urinary sediment has been found unsatisfactory for the detection of pyuria (16-19). The introduction by Hamburger et al. (8) and Houghton and Pears (9) of

simple and reliable methods for quantitative determination of leucocyte excretion has made it possible to make a more exact diagnosis of pyuria.

Quantitative methods require estimation of the leucocyte concentration using a counting chamber. It has been shown that the coefficient of variation in counting increases as the number of cells per mm³ decreases (5, 19, 24, 29). In order to reduce the error in counting, several authors have concentrated the specimens by centrifugation and resuspended the deposit in one tenth of the original volume. It has been demonstrated that centrifugation and presumably also the resuspension are inadequate and the leucocyte concentration thus obtained will be too low (4, 29).

Another problem is the differentiation of non-squamous epithelial cells and leucocytes (27). The two types of cells are of nearly the same size but non-squamous epithelial cells vary more in shape, are often elongated and angular (15). Leucocytes are easier to recognize after staining with Sternheimer-Malbin's stain (30) or after staining according to Prescott and Brodie (26).

In the present study, good correlation was found between the leucocyte concentration in urine aspirated by suprapubic puncture and in mid-stream specimens. In a similar comparison of specimens from 12 puerperal women, Roberts et al. (28) found considerable deviations in some of the patients. It is possible that the discrepancies have to some extent been due to the great risk of contamination from vaginal discharge in these patients.

In order that the diuresis may be determined within a short time, the importance of complete emptying of the bladder is obvious. To ensure this as far as possible without catheterization, determination of the diuresis was based on two

Table II Results of microscopic examination of urinary sediment in relation to 46 reinfections in 44 patients. All these patients had a period of at least two months with sterile urine prior to the last urine examination before the reinfection.

	Before reinfection	Reinfection		After start of treatment		
		1st specimen	2nd specimen	1 week	2 weeks	4 weeks
Max. no. leucocytes/HPF						
0-2	39	10	11	32	32	35
3-5	6	8	12	5	3	3
6-20	1	16	12	5	5	2
>20	0	12	10	0	1	0
No. of specimens	46	46	45	42	41	40

consecutive voidings without the patients being instructed to urinate at a predetermined time

Healthy subjects have been found to excrete fewer than 400 000 leucocytes per hour (4 9 10 16 24). A slightly higher upper limit was found by Prescott (27). It has been found that women have a higher excretion rate than men (16 27). No correlation between age and excretion rate has been demonstrated (9).

Pears and Houghton (25) and Osborn and Smith (24) have found the rate of excretion to be rather constant in the individual patient while Montgomerie and North (23) and Prescott (27) have found extreme variation in some subjects. Only minor variation between diurnal and nocturnal excretion rate has been demonstrated (6 27). Very little is known about the influence of urine flow on the leucocyte excretion (7 25).

Pyuria is defined as excretion of more than 400 000 leucocytes per hour. The diagnosis of pyuria may be made by quantitative determination of leucocyte excretion. However the urine of nearly all patients with pyuria contained more than 10 leucocytes per mm^3 while the presence of 10 or fewer leucocytes per mm^3 is seldom associated with pyuria. It must be mentioned that these studies were performed on urine specimens collected during periods of low urine flow. Comparison of the excretion rate and the number of leucocytes seen per HPF by microscopic examination of the urinary sediment has shown that three or more leucocytes per HPF indicate pyuria while fewer leucocytes per HPF do not exclude pyuria (16 19).

Pyuria in bacteriuria

As in other studies (12 31) it was found that ordinary microscopic examination of the urinary sediment revealed pyuria in only about seventy per cent of the patients with bacteriuria. Among the women with urinary tract symptoms but without bacteriuria more than three leucocytes per HPF were found in 38%. It must be considered that pyuria in some of these patients had been caused by chronic pyelonephritis with temporarily sterile urine or chronic glomerulonephritis (3). Others may have had urinary tract infection with fewer than 10 000 bacteria per ml in two consecutive specimens (20) or have spontaneously attained sterile urine before the examination. Twelve of the patients had been treated with chemothera-

peutics or antibiotics within the last week before the examination.

Microscopic examination of urinary sediment also proved an unsatisfactory means of detecting remission.

In patients with more than 10 bacteria per ml urine Fairley and Barreclough (3) found an excretion rate of more than 300 000 per hour in 93% of 117 patients examined. Osborn and Smith (24) more than 200 000 per hour in all of 19 patients and Little (17) more than 200 000 per hour in 72% of 265 pregnant women. In these studies leucocytes were counted after centrifugation of the specimens. Mond et al (22) found more than 10 leucocytes per mm^3 uncentrifuged urine in all of 38 patients with bacteriuria.

In the present study 144 (95%) of 152 women with bacteriuria excreted more than 400 000 leucocytes per hour. Whether in the remaining eight patients the bacteriuria was present without invading or causing inflammatory changes in the tissue of the urinary tract, cannot be determined. However the fact that signs of inflammation were found in 95% of the patients with bacteriuria supports the generally accepted view that bacteriuria indicates infection in the urinary tract (14).

The magnitude of leucocyte excretion was found to be independent of the type of bacteria isolated from the urine. This strongly supports the view that coagulase negative staphylococci are pathogenic in the urinary tract (21).

Impaired urine concentration ability in bacteriuric patients must be regarded as a sign of pyelonephritis (11 32). The leucocyte excretion rate was found to be lower in patients with normal concentration ability i.e. over 750 mOsm/kg (2). Leucocyte excretion seems to be higher in patients with upper urinary tract infection than in patients with lower urinary tract infections. On the other hand Hutt et al (10) have found that only six of 14 patients with acute or chronic pyelonephritis and "positive urine culture" excreted more than 10⁴ leucocytes per hour.

It must be concluded that simple microscopic examination of urinary sediment is an unsatisfactory means of revealing pyuria. If pyuria is defined as the excretion of more than 400 000 leucocytes per hour a few patients with bacteriuria may not have pyuria but this does not exclude the possibility of inflammation in the urinary tract. For all practical purposes bacteriuria must be

regarded as an indication of urinary tract infection
As a consequence of this examination for pyuria
is of lesser significance if bacteriuria has been
demonstrated

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PULMONARY HAEMOSIDEROSIS AND GLOMERULONEPHRITIS

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Abstract Three cases with the combination pulmonary haemosiderosis and glomerulonephritis (Goodpasture's syndrome) are described. All the patients died. A temporary improvement was seen in two cases with steroid treatment. In one of the patients the disease appeared in two distinct bouts. In the first she had a localized lung process and microscopic haematuria. After lobectomy the state was normalized but one year later she had a rapid deterioration with pulmonary haemorrhages and glomerulonephritis.

The syndrome described in 1919 by Goodpasture (6) consists of acute glomerulonephritis and pulmonary haemosiderosis. In medical publications from Scandinavian countries cases have been reported from Norway (14), Denmark (19) and Finland² (20). No case has as far as we know been reported from Sweden.

Idiopathic pulmonary haemosiderosis as a diagnostic entity is known since the 19th century when it was described by Virchow (21). The first time the disease was diagnosed *in vivo* was by Waldenström (22, 23) in 1940. Since then the number of cases published exceeds several hundreds. Goodpasture's syndrome (lung haemorrhage and glomerulonephritis, lung purpura with nephritis, pulmonary haemosiderosis and nephritis, haemorrhagic and interstitial pneumonitis with nephritis, the haemorrhagic pulmonary renal syndrome) is very vaguely defined and we think it is justified to describe three cases.

CASE REPORTS

Case 1

L. R., a 28-year-old woman who fell ill Nov. 1957 with acute tonsillitis. She was treated for eight days with penicillin. After that she had several relapses of tonsillitis for

a month or more. Also signs of swolleness of the knees were noted. The antistreptolysin titre (AST) was 1000 IU and fell after treatment to 210 IU. There are no reports of urinary analysis on this occasion. During the winter of 1959 the patient had several attacks of sore throat and on Febr. 9, 1960, red blood cells were noted for the first time in the urinary sediment. During this time the patient complained of increased nasal discharge. She was then given penicillin but because of generalized pain and palmaritch the penicillin was stopped and chloramphenicol was instituted. On Febr. 29 she was a bit feverish and at the same time haemolytic streptococci type A III were isolated from her throat.

First admission. On Mar. 1, 1960, the patient was admitted to the Department of Internal Medicine. She was pale and there was a notable sign of transparency in her face. There were no signs of cardiac decompensation or jaundice. Blood pressure 135 mm Hg systolic and 75 mm Hg diastolic. The urinary sediment contained several red blood cells, NPN was 45 mg%, Hb 90 g, ESR 110 mm, haaptoglobin 212 m_u and serum iron 32 µg%.

On Mar. 2, 1960, she suddenly had a serious haemoptysis. X-ray showed in the middle part of both lungs rather symmetrical very widespread densities with diffuse borders against a normal upper and basal lung parenchyma. In the densities an irregular mottling could be defined from smaller opacities of varying size. The picture corresponded well with that of recurrent pulmonary haemosiderosis. During a fortnight there was a period of improvement interchanging with short relapses. On April 16, 1960, the lung picture was definitely normal.

The patient was dyspnoeic even at rest and had a pulse rate of 110/min. On the tentative diagnosis of pulmonary haemosiderosis steroid treatment (triamcinolon) was instituted and the patient underwent immediate improvement. On Mar. 15, the serum creatinine was 3.16 mg%. The steroid dose was at that time lowered to 8 mg triamcinolon/day and the patient's condition deteriorated. As before the several red blood cells in the sediment. The Hb was 78 g and the ESR 105 mm. The patient thus had a new relapse of her pulmonary haemorrhage and the steroid dose was again increased to 16 mg/day. On this treatment she again improved. The definite regression of the X-ray findings in the

Second admission On Sept 4 1960 the patient was admitted to the Department of Infectious Diseases because of acute gastroenteritis. The Hb was at that time 51 and there were a few red blood cells in the urinary sediment. Serum electrophoresis showed a slight increase of alpha 2 globulins but was otherwise normal. The patient was discharged improved.

Third admission On Febr 5 1961 the patient was again admitted to the Department of Infectious Diseases after she had fallen ill with headache vomiting and a feeling of swollenness in her body. At admission her blood pressure was 200 mm Hg systolic and 111 mm Hg diastolic. The Hb was 45, the ESR 111 mm and the urinary sediment contained no red blood cells. The serum creatinine was 7.7 mg. An X-ray of the chest showed minor relapses of the changes from April 1960.

Because of the renal insufficiency steroid treatment was considered contraindicated. On Febr 19 a tender gland was noted in the right submandibular space and penicillin treatment was started. On Febr 22 the patient was transferred to the Department of Internal Medicine. Here a harsh systolic murmur was heard all over the precordium. The blood pressure was 180 mm Hg systolic and 120 mm Hg diastolic. The urinary output was low. There were also signs of pericarditis, pleuritis and evasive arthritis.

On the chest film (bedside) enlargement of the heart and fluid in both pleural sinuses was seen but no parenchymal process of haemosiderosis type. The patient was given Actocortin intravenously but without improvement.

On Mar 1 she was transferred to the Haemodialysis Centre in Lund where a dialysis was performed on Mar 1. The patient died after 5 hours of treatment.

Pathological findings Terminally the patient was treated in a clinic for kidney disease at another hospital and was autopsied there. According to the report small kidneys were found: the right kidney weighed 80 g, the left kidney 100 g. The surfaces were brown red, coarsely granulated with several small haemorrhages. The cortex was slightly reduced.

The right lung weighed 380 g, the left lung 320 g; they were heavy with increased density and from the cut surfaces especially in the upper lobes abundantly foamy pink-coloured fluid poured out.

Microscopically most of the glomeruli were hyalinized with diffuse interstitial infiltration of lymphocytes and plasma cells. Only a few intact glomeruli were seen and within these there were adhesions between the capsule and the capillary tufts. Also scattered crescent-shaped epithelial proliferations were seen in the capsule. There were no signs of vasculitis.

In the lungs there were round lesions where the septa and alveolar walls were fibrotic containing a large number of iron pigment loaded macrophages. There were also large irregular areas with fresh bleedings and several macrophages containing haemosiderin.

Case 2

N B E, an 8-year-old boy who had previously been healthy fell ill on Aug 2 1961 with fatigue, sore throat and a slight rise of temperature.

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First admission On Aug 14 dark-coloured urine was noted and the patient was admitted to the Pediatric Clinic at Malmö General Hospital. There were several red blood cells in the urinary sediment and albuminuria. The Hb was 49, red blood cells 2.4 mill, ESR 60 mm, and serum iron 23 µg. Blood pressure was 170 mm Hg systolic and 75 mm Hg diastolic. The preliminary diagnosis was acute glomerulonephritis. The patient complained however of cough and shortness of breath even after slight exercise. During the following days the blood values fell and on Aug 25 the Hb was 31.

X-ray of the chest Aug. 16. In both lungs rather dense widespread mostly peribronchial and basal parenchymal infiltrates more on the right than on the left side. The apical regions seemed to be free. The infiltrates were rather diffuse peripherally and more patchy near the hilar regions. They did not respect the borders between the cortex and the central parts of the lungs, speaking against a uraemic cause. The picture corresponded well with that of acute pulmonary haemosiderosis.

The patient was treated with corticosteroids initially 30 mg of prednisolone daily. The AST was 360 IU. During the whole time there was haematuria with 0-30 red blood cells in the urinary sediment and also granular and hyaline casts. The patient was discharged improved on Oct 25 1961.

Second admission On July 5 1962, the patient was again admitted to the Pediatric Clinic because of abdominal pain, increasing fatigue and dark coloured urine. X-ray of the chest was at this time normal. Haemolytic streptococci were found in the throat. The AST was 600 IU. The patient was for a short time treated by elimination of milk products (7). There was no improvement on this treatment. Instead the patient was given corticosteroids, improved again and was discharged in a fairly good condition on Aug 10 1962. The Hb was then 53% and the serum electrophoresis showed a slight increase in alpha and gamma globulins.

Third admission On Oct 10 1962 the patient's condition deteriorated. He experienced shortness of breath and had several haemoptyses. On X-ray a typical relapse of an acute pulmonary haemosiderosis was seen.

The patient was again treated with corticosteroids, starting with 30 mg prednisolone. During this treatment there was definite regression of the lesions and the patient was discharged improved on Dec 19. The Hb was then 51% and serum iron 43 µg.

Fourth admission On X-ray examination of the chest on Jan 17 1963 there were signs of relapse. Therefore the patient was again admitted to the Pediatric Clinic. There were no catarrhal symptoms but the patient had vomited several times during the last day and was complaining of slight abdominal pain. On this occasion too prednisolone treatment was started 30 mg daily. During this treatment the patient improved. He was discharged on continued steroid treatment.

Fifth admission On April 15 1963 the patient was again admitted to the same clinic. During the last day he had vomited quite a lot and these vomits had been blood.

tinged. In connexion with this his prednisolone dose was increased so that at admission it was 30 mg daily. The patient now had manifest oedema and hypertension 165/130 mm Hg. Because of this hypertension he was treated with alphanemethyl-dopa.

The Haemodialysis Centre in Lund was consulted but there was no indication for haemodialysis at this time. The patient's Hb was 77. On May 4 it had fallen to 46.

In July 1963 the patient was on holiday in northern Sweden. On his way home he was admitted to the Children's Hospital in Sundsvall where he had some serious haemoptyses which in the course of a few days killed him.

On admission to the Children's Hospital general malaise, dyspnoea with rales over the lung bases and a typical Cushingoid picture was noted.

Pathological findings. At autopsy the organs had been placed in a small amount of formalin without previous dissection and because of this the quality of the preparations was somewhat inferior.

Microscopically large bleedings as well as lots of macrophages containing iron pigment were seen in the lungs. The alveolar walls were somewhat thickened but there was only slight fibrosis. In many areas the alveoli contained exudate, polymorphonuclear leukocytes and fibrin.

In the kidneys most of the glomeruli were hyalinized. In some glomeruli, however, capillaries were seen which showed a high degree of hyalinization of the wall and in some places there was a proliferation of the capsular epithelium with crescent formation. Many tubules were destroyed to a high degree and replaced by fibrous tissue. There were no signs of vasculitis.

Case 3

M. L. a 50-year-old unmarried children's nurse who because of her work, was controlled once a year with fluoroscopy of her lungs. The patient fell ill on May 72 1963 with fever. Fluoroscopy of the lungs which was performed on May 27 showed rounded lesions basally on the right side. The patient was then treated with penicillin because of fever and cough with no effect on the temperature and therefore the treatment was changed to tetracycline. This treatment had no effect either on her fever. The patient had on this occasion had no haemoptysis.

First admission. The patient was admitted to the Department of Lung Diseases, Malmö General Hospital on June 14 1963. At this time she had signs of general malaise and was distinctly pale. There were no physical signs from the lungs. Because of a positive Mantoux reaction, treatment with PAS, INH and streptomycin was instituted on June 17. The X-ray changes remained unchanged on the right side and therefore a bronchoscopy was performed on July 2 which showed a slight irregularity in the mucous membrane of the right lower lobe. No biopsy was taken.

Bronchography, right lung. The bronchi of the upper and middle lobes were normal. In the lower lobe the

bronchi of the apical segments were somewhat dislocated in a caudal direction. Some small ramifications showed small mucosal intraluminal irregularities. There were no signs of a process starting on the bronchial system.

Several cytological examinations of sputum showed no signs of malignant cells but in one specimen cell atypia was noted. Furthermore a few macrophages containing haemosiderin were seen.

Other examinations and laboratory tests. X-ray urography normal. Guinea pig inoculation test three times negative. AST 125 IU, CRP 20 mm. Urinary sediment showed 10-12 red blood cells. The Hb was 73 g and the ESR 108 mm.

Spirometry showed decreased function of the left lung and this finding was interpreted as a restrictive lung disease of moderate degree.

On July 4 the patient was transferred to the Clinic of Thoracic Surgery where on July 10 a lobectomy of the lower lobe of the right lung was performed. The lobectomy was without complication and the patient was discharged from the hospital on July 24 in good condition.

Pathological findings. Examination of the surgery specimen showed a rounded and fairly well defined tumour of 4 cm diameter in the right lower lobe. The consistency was soft and the cut surface greyish white.

Microscopically the normal alveolar structure was totally lost in the central parts and replaced by collagenous fibrous tissue containing several lymphocytes, plasma cells and scattered eosinophilic polymorphonuclear leukocytes. Furthermore irregular giant cells of foreign body type were seen but no epithelioid cell granuloma. No anastrophic material was seen (Fig. 1). The walls of some vessels were thickened and in a few places inflammatory cells were seen in the wall. However, there were no fibrinoid necroses and the picture was in the first place consistent with a secondary vascular engagement and not a primary vasculitis. In the periphery of the lesion markedly thickened alveolar septa were found, surrounding small cavities. The septa were mostly compact and rich in collagen but in some places, especially in the periphery they were loose and contained some fibroblasts. In PAS (periodic acid-Schiff) staining, no real basal membranes were seen, only faintly stainable slightly split-up material. The elastic fibrils were of normal appearance both in the alveoli and in the fibrous tissue. A large number of haemosiderin-containing macrophages were found and in some places also groups of macrophages containing fat positive substance. The findings did not, however, correspond with those of lipid pneumonia. Tubercle bacilli, fungi or other parasites were not present.

The lesion was interpreted as a chronic inflammatory process of unknown nature.

The patient visited the Clinic of Thoracic Surgery on several occasions and on May 5 1964 a microscopic haematuria was noted, a finding which had been noted also at the examinations in July 1963.

Second admission. On August 9 1964 the patient fell ill with high fever, general malaise, soreness in the eyes and muscle pain. On August 1 there was an acute tonsillitis.

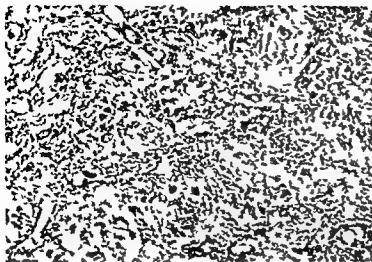


Fig 1 Central part of surgical specimen of lung with fibrosis inflammatory reaction and multinucleated giant cells Haematoxylin-eosin $\times 160$

and the patient was treated with penicillin. In spite of this a typical erysipelas appeared on Aug 25 on the nose and cheeks and the temperature was above 38°C. On Aug 27 there was an erythematous multiform-like rash in the left palm and lower arm. The treatment was changed from penicillin to oxytetracycline and on Sept 2 the patient was afebrile and the rash was gone.

Third admission. The patient was admitted to the Department of Infectious Diseases on Oct 5 1964 after having been subfebrile for 14 days with a temperature around 38°C, headache and conjunctivitis. A tender lymph node as also found in the right submandibular space. The ESR was 88 mm and the Hb 65 g/l. On admission it was noted the patient seemed illigued and very pale.

X-ray of the chest on Oct 13 1964. Small right-sided operative pleural adhesions as in earlier examinations this year. (Right lower lobe was resected on July 10 1963.) In the basal posterior part of the right lung dense widespread infiltrates were observed. In the perihilar region the changes had a more mottled appearance with small opacities of varying size. No changes in the left

lung. The changes corresponded well with a pneumonia.

Three days later a new chest radiogram revealed a progress of the parenchymal changes also in the subapical parts of the right lung. Still no changes in the left lung. The heart was of normal size and shape. The pulmonary vessels were normal. Pneumonia was still the most probable radiological diagnosis.

On Oct 16 penicillin treatment was instituted and the patient's condition then grew worse. She was more pale, there was a new process in the right lung and the patient had daily haemoptyses.

The Hb was 7.6 g, NPN 40 mg, serum creatinine 1.6 mg/dl. Macroscopical haematuria with both haemaline and granular casts was noted. The ESR was 100 mm, serum iron 35 µg/dl, and TIBC 232 µg/dl.

During her first stay in the Department of Infectious Diseases the patient was treated with penicillin and chloramphenicol. No corticosteroid treatment was given. She was transferred to the Department of Internal Medicine with the presumptive diagnosis of systemic lupus erythematosus.

During the stay in the Medical Clinic the patient was



Fig 2 Irregular fibrous thickening of alveolar walls. Large amounts of red blood cells and haemosiderin-containing macrophages. Haematoxylin-eosin $\times 128$

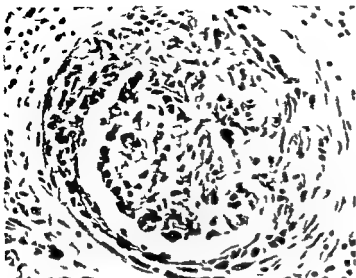


Fig 3 Glomerulus with epithelial proliferation and crescent formation. Haematoxylin-eosin, $\times 350$.

treated with oxytetracycline. Again steroid treatment was given. During the whole course the patient was subfebrile.

The blood pressure was 130/80, the Hb 63 g, the total eosinophil count 944, serum iron 54 μg , TIBC 247 μg , ESR 145 mm, haptoglobin 350 mg% and serum creatinine 3.45 mg%. Several red blood cells were seen in the urinary sediment. The ANF was negative. No LE cells were found. The AST was 360 IU.

The patient grew still worse with nausea, vomiting, increasing uraemia, tendency to pulmonary oedema and daily haemoptyses. She died on Oct. 10, 1964.

Pathological findings. Besides a fatty liver of slight degree there were changes only in the lungs and the kidneys. The lungs were solid, especially on the right side (the right lung weighed 610 g, the left lung 650 g). The cut

surfaces showed dark reddish blue irregular areas with an increased amount of foamy fluid. On both sides small apical fibrous scars were seen.

The kidneys had normal fat capsules (the right kidney weighed 110 g, the left kidney 130 g). The fibrous capsule was rather easily removed and the kidneys had a yellow brown surface with dilated vessels and petechial haemorrhages. The cut surface showed a cloudy picture with an unsharp border between cortex and marrow. The colour was greasy yellow brown. The pelvis and calyces were normal with pale mucous membrane.

Microscopically the lesions of the right and left lungs were similar although more pronounced on the right side. The picture was dominated by areas with large amounts of red blood cells in the alveoli. Furthermore many

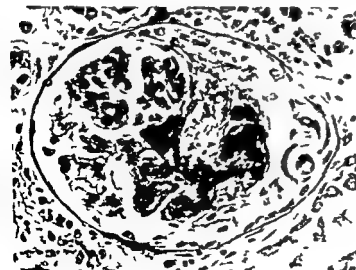


Fig 4 Glomerulus with fibrinoid necrosis in some of the capillary loops. Phosphotungstic acid haematoxylin, $\times 400$.



Fig 1 Central part of surgical specimen of lung with fibrosis inflammatory reaction and multinucleated giant cells Haematoxylin-eosin $\times 160$

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A chest X-ray on Oct 13 1964. Small right-sided postoperative pleural adhesions as in earlier examinations this year. (Right lower lobe was resected on July 10 1963.) In the basal posterior part of the right lung dense widespread infiltrates were observed. In the peripheral region the changes had a more mottled appearance with small opacities of varying size. No changes in the left

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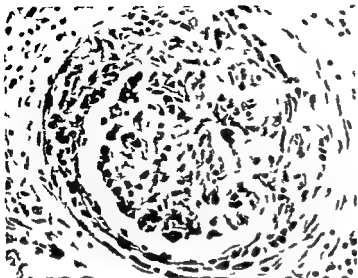


Fig 3 Glomerulus with epithelial proliferation and crescent formation. Haematoxylin-eosin, $\times 360$

treated with oxytetracycline. Again steroid treatment was given. During the whole course the patient was subfebrile.

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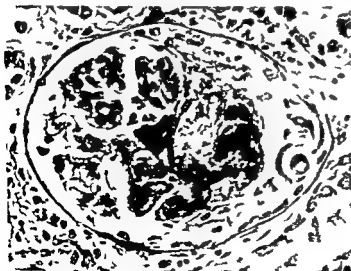


Fig 4 Glomerulus with fibrinoid necrosis in some of the capillary loops. Phosphotungstic acid haematoxylin, $\times 400$

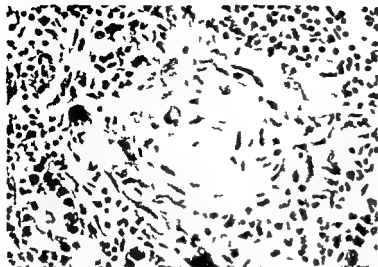


Fig 5 Destroyed glomerulus leaving fibrous scar with giant cells Haematoxylin-eosin, $\times 360$

haemosiderin containing macrophages were seen both in the alveoli and in the alveolar walls many of which showed fibrous thickening. The fibrosis was of the same type as that in the peripheral parts of the surgical specimen (Fig 2). Compact collagenous parts were not present. No giant cells. No fat positive substance. No signs of vasculitis but in several medium-sized vessels thrombi were seen in some parts in organization. Furthermore bronchopneumoniae were seen even in parts without haemorrhage. The inflammatory cells were mostly inside the alveoli. With PAS staining no distinct basal membranes were seen in the alveolar walls only diffuse patches of lightly stained material. There was no splitting up of the fibrils.

In the kidneys there were more or less pronounced lesions in practically all glomeruli. A few were apparently normal and a few showed prominent epithelial prolifera-

tion with crescent formation (Fig 3). In some there was fibrinoid necrosis involving only one or two capillary loops while in others more or less the whole tuft was necrotic. The necrotic material was strongly stained in PAS and PTAH (phosphotungstic acid haematoxylin) (Fig 4). The necrotic tufts had been partly or totally replaced by fibrous tissue. Some were hyalinized but the destruction in most glomeruli resulted in small and vaguely demarcated fibrous scars containing fibroblasts and a few giant cells (Fig 5). In some of these scars small irregular rests of necrotic capillaries were seen (Fig 6). Tubuli were in some parts atrophic and some tubuli showed a hyaline droplet degeneration (Fig 7). Intersitally there were patches of inflammatory cells both plasma cells, lymphocytes and polymorphonuclear leucocytes. There were no vascular changes except those described in the glomeruli.

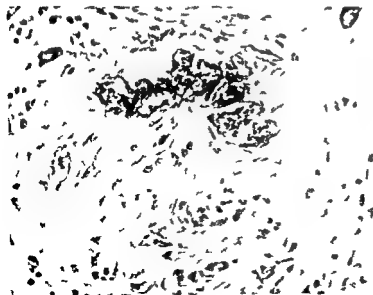


Fig 6 Scar with irregular rests of necrotic capillaries. Giant cells in the periphery. Periodic acid Schiff $\times 400$



Fig 7 Tubulus with hyaline droplet degeneration. Periodic acid Schiff $\times 400$

DISCUSSION

The lung lesions in Goodpasture's syndrome can not be separated clinically, radiologically or pathologically with certainty from those seen in idiopathic pulmonary haemosiderosis (IPH) a disease which may be complicated by glomerulonephritis. Heptinstall and Salmon (10) studied 69 cases of IPH and found five with histologically proved kidney lesions of focal glomerulonephritis type. In one of these cases there were changes similar to those found in periarthritis nodosa (PAN). They also found three cases with clinical signs of kidney disease but data of the kidneys were lacking in the autopsy records. It is very unlikely for many reasons that all cases with the combination of pulmonary haemorrhage and glomerulonephritis would be an accidental coincidence of IPH and glomerulonephritis. The combination is more common than to be attributable to mere chance and IPH is predominantly a disease of children of both sexes while Goodpasture's syndrome is usually seen in younger men. In most of the cases of Goodpasture's syndrome when kidney biopsies have been performed the glomerulonephritis in the earlier stages has been found to be focal and local but in a very short time it may become diffuse in patients who live long enough (1, 9, 11, 17, 18). Such focal nephritis is often seen in diseases which are considered to be of autoimmune type e.g. collagenoses (PAN, systemic lupus erythematosus and Schönlein-Henoch's purpura) but these diseases do not have the same distribution in sex and age as Goodpasture's syn-

drome. In the so-called recurrent haematuria or isolated haematuria a focal glomerulonephritis is found and this disease has the same age and sex distribution as Goodpasture's syndrome. In these cases however the disease starts with a haematuria which is usually profound and the attacks return at intervals of weeks, months or years. Proteinuria is not a constant finding but may develop after several attacks. This disease often starts in connection with or immediately after an infection usually in the upper respiratory tract and the interval which is typical of the classical post-streptococcal glomerulonephritis is missing. No definite relation to a streptococcal infection has been proved and a clinical picture of this type is not commonly found in Goodpasture's syndrome.

It has been suggested that a streptococcal infection should be the cause of Goodpasture's syndrome (15, 20). Other authors (17) do not think this likely. They do not find anything specific at kidney biopsy and the picture is not similar to that usually seen in post-streptococcal glomerulonephritis.

Benoit et al. (1) have proposed autoimmunity as the cause of Goodpasture's syndrome and that this autoimmunity might be provoked by a viral infection. They base this suggestion on the case described in 1919 by Goodpasture, a young man who suddenly fell ill with pulmonary haemorrhage and glomerulonephritis after an attack of epidemic influenza. The other fact put forward by Benoit et al. (1) is that most of the cases of Goodpasture's syndrome described started in the years 1957 and

1958 when there was a simultaneous pandemic of influenza. There is however no definite proof of autoimmunity started by a viral infection. Cultures from three patients have yielded no viral growth and no viral antibodies have been found. Duncan et al (5) demonstrated viruslike bodies by electron microscopy but Rohr et al (17) were not able to confirm this. Lexow and Sigstad (14) found no antibodies to kidney tissue. Experimentally however it has been shown that lung antibodies might give rise to nephritis through a cross reaction (4) and common antigens in both epithelial and mesenchymal basal membranes in different tissues have been demonstrated (12, 13). According to some authors (1, 17) cases which show inflammatory changes of the vessels should not be considered as Goodpasture's syndrome. According to Heptinstall (8) however they should belong to this group. He states that there is no sharp limit in the histological picture between cases with and without vascular changes and in the case of Goodpasture's syndrome from 1919 vascular lesions were found in the gut and necroses were seen in the spleen in connexion with the blood vessels.

The histological picture has been described in the case reports. In two of the cases the kidney lesions were very advanced and showed the picture of a diffuse glomerulonephritis with practically all glomeruli hyalinized. Only a few glomeruli were somewhat better preserved but in these as well there was hyalinization in most of the tufts. Adhesions were seen. In some places there were epithelial proliferations with crescent formation. In case 3 however focal glomerulonephritic changes were seen in different stages and of the same type as that described by other authors in early stages of Goodpasture's syndrome. Benoit et al (1) considered the description fibrinoid necrosis of the lesions in the capillary tuft incorrect because they did not have the tinctorial properties of fibrin (PAS and PTAH staining). These stainings were however positive in our case 3 the only case which also had earlier changes at autopsy. In this case too foreign body giant cells were seen around destroyed glomeruli. This has earlier been described by Cruickshank and Parker (3). None of the three cases showed inflammatory vascular changes.

The pulmonary lesions were of the type described in IPH. Thus scattered intra alveolar haemorrhages were found and several haemo-

siderin containing macrophages in the sputa as well as in the alveoli. In all cases there was a fibrous thickening of the alveolar septa most pronounced in case 3 who had had a localized process in the right lung one year earlier. In all cases pronounced inflammatory changes of the type terminal bronchopneumonia were found. No necrotizing alveolitis as described by Parkin et al (16) was found. Elastase stained sections did not show any splitting up and there were no signs of vasculitis. In PAS stained sections no distinct basal membranes were found in the alveolar septa. However patches of vaguely stained PAS positive material were seen in the alveolar walls. Rohr et al (17) demonstrated similarities in the lesions of kidneys and lungs. The lesions were localized predominantly on the capillary side of the basal membrane. They suggest that the lesion has a great similarity to antigen antibody reaction. Case 3 is of special interest because of the unusual course which gives the impression of a case of Goodpasture's syndrome appearing in two distinct bouts but with some differences both clinically, roentgenological and histopathological. As a sign of kidney affection there was in the first bout only a microscopic haematuria although the kidney function was normal. The lung lesion was characterized by a localized process in the right lower lobe. Histopathologically there was a pronounced fibrosis with multinucleated giant cells of foreign body type. Otherwise the changes were of principally the same type as at autopsy one year later. Clinically there were no haemoptyses but both in sputum and in the surgical specimen haemosiderin containing macrophages were seen. Furthermore macrophages were seen containing fat positive substance but the picture had no similarity to lipid pneumonia in other aspects. A similar picture has been described by Botting et al (2). On electron microscopic examination of lung tissue they found material which was thought to be lipid or lipoprotein. The cause of this localized haemosiderosis is not clear. After lobectomy the haematuria disappeared. And if one considers Goodpasture's syndrome to be an autoimmune process it is possible that by removal of changed lung tissue the production of antibody was stopped or depressed and at least for a while retarded the progress of the disease.

As the aetiology and pathophysiology of Goodpasture's syndrome is not known in detail there is

no rational treatment. In the literature there are reports of cases who have survived the disease but most have died. Some authors claim that steroid treatment is the treatment of choice because the disease is considered to be an immunopathy. A temporary improvement connected with steroid treatment was seen by us in both case 1 and case 2.

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PERNICIOUS ANAEMIA PARAPROTEINAEMIA WITH UNUSUAL FEATURES AND CHROMOSOME ABERRATIONS

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Abstract A case of paraproteinaemia associated with pernicious anaemia is described. The patient's serum contained 2 M-components (IgM and IgG) while the urine contained gamma globulin of the L-chain type. All abnormal components belonged to type Kappa. Chromosome studies on blood cultures revealed a mosaic pattern about one third of the cells having a karyotype identical to that found in Down's syndrome (trisomy of a small acrocentric chromosome) the remainder having a normal male constitution.

While a megaloblastic erythropoiesis has occasionally been described in myeloma patients (13) true pernicious anaemia appears to be rare in this condition. Mandema (24) reported one case and Larsson (20) four cases. In two instances myeloma and pernicious anaemia were observed in the same family (18, 23). No reports on the coexistence of pernicious anaemia and Waldenström's macroglobulinaemia have been found in the literature.

In some cases of paraproteinaemia unusual features have been described for example the presence of more than one M-component in serum (9, 19) or chromosome aberrations (4).

The purpose of this paper is to report a case of pernicious anaemia exhibiting two different M-components in serum, excretion of light chains in the urine and chromosome aberrations.

MATERIAL AND METHODS

1 Case History

The patient was a married gardener born in 1889. The patient could give no information concerning cases of blood dyscrasias or chromosome abnormalities, especially Down's syndrome, in the family.

When he was 73 years old he (V H) was admitted on account of general lassitude, pain in the tongue, acro-

paraesthesiae and a loss of weight of ten kg in six months. On admission the patient appeared pale and emaciated. The tongue was smooth and red, but uvula and soft palate were normal. No jaundice was observed. Patellar and Achilles-tendon reflexes as well as vibration tests were normal. Lymph nodes, liver and spleen were of normal size. Bone tenderness, bone protuberances, purpura or Raynaud phenomena did not occur.

Paraproteinaemia and pernicious anaemia were diagnosed. Antipernicious treatment was started with a vitamin B₁₂ depot preparation (Betolvek®) 1 mg i.m. once a week for five weeks. Thereafter the patient was given vitamin B₁₂ i.m. every 4th week. After treatment for eight weeks the haemoglobin concentration was normal. The patient's paraproteinaemia was not treated.

The patient was controlled nine times during 3 1/2 years. His general condition remained good and haemoglobin levels have been normal. No clinical signs of multiple myeloma or Waldenström's macroglobulinaemia have been detected although the ESR has consistently been elevated and the paraproteins in blood and urine have been unaltered.

Laboratory tests

Blood: W.R. neg., Hb 6.5 g/100 ml, ESR 151 mm/h, erythrocytes 157 mill./ μ l, colour index 1.43, thrombocytes 240 000/ μ l, leukocytes 4 360/ μ l, the differential count showed a slight lymphocytosis. The neutrophils were hypersegmented. The red blood picture showed megalocytosis and a marked anisocytosis and poikilocytosis. No plasma cells were found in peripheral blood. Serum B₁₂ level was 60 pg/ml, serum folic acid 0.008 μ g/ml, serum iron 146 μ g/100 ml and serum TIBC 304 μ g/100 ml.

Urine: No protein could be detected by conventional methods. Tests for glucose, blood and urobilin were negative. Amylase 3 units. Xylose test 10.4 g was excreted during 4 h after ingestion of 75 g xylose. Schilling test without intrinsic factor: urinary excretion 0% of the radioactive B₁₂ dose. Schilling test with intrinsic factor showed a 4.0% excretion.

Faeces: Eight samples gave negative benzidine reaction. Gastric juice: Negative Congo reaction 60 min after histamine injection.



Fig 1 (a) Abnormal plasma cell with flaming of the cytoplasm ($\times 1400$) (b) Abnormal plasma cell with thesauric appearance eccentric somewhat prominent nucleus and large homogeneous cytoplasm divided into compartments by the basophilic trabeculae ($\times 1400$)

Biopsy from mucous membrane of rectum. No sign of amyloidosis (sign Prof G Teitum)

Retina Retinopathy paraproteinaemia

(a) examinations No osteolytic lesions or diffuse halisteresis were found either in the cranium costae columna pelvis or the long bones Intravenous urography and examination of colon with contrast showed normal conditions Heart and lungs were normal but a slight atherosclerosis was noted in the aorta. Examination of jejunum and ileum with contrast showed a slightly flocculent appearance

Other routine laboratory studies Serum bilirubin 0.4 mg/100 ml prothrombin proconvertin time after Owren 85% serum sodium 147 mEq/l serum potassium 4.7 mEq/l serum chloride 100 mEq/l standard bicarbonate 24.7 mEq/l serum creatinine 0.7 mg/100 ml serum glutamic-oxalacetic transaminase alkaline and acid phosphatase all showed normal values LE-cell phenomenon did not occur serum cholesterol 17 mg/100 ml ECG normal

Sternal marrow Cell content 360 000 μ l Megaloblasts

and many giant stabs were found Only 2 plasma cells were present several plasma cells showed flaming cytoplasm (Fig 1a) Few thesaurocytes (Fig 1b) were observed Differential count revealed 70 lymphocytes and 2 reticulum cells Many of the lymphocytes had very little cytoplasm but seemed otherwise normal (sign Prof G Gormsen)

Protein examinations Total serum protein 8.3 g/100 ml Paper electrophoresis showed an M-component with gamma mobility contributing 19.5% of the total protein.

Immunoelectrophoresis (IE) IE was performed as described by Clausen (7) In all cases 1.5 μ l antiserum sample was used In all studies 80 μ l antiserum were used (vide infra) Two paraproteins were present an IgG and an IgM type (Fig 2a) The IgM paraprotein precipitated weakly with anti IgA antiserum

2 Methods for Chromosome Investigations

Chromosome preparations were made from short term cultures from peripheral blood on two occasions. The

method was essentially that described by Moorehead et al (6). Skin cultures were also studied (14). An attempt to study the chromosomes in a direct preparation from bone marrow was unsuccessful.

3 Methods for Protein Investigations

The paraproteins present in serum (diluted initially twice with distilled water) and urine (concentrated 100 times in vacuum dialysis) were prepurified by stepwise ammonium sulphate precipitation (pH 6.8) and isolated by Sephadex G 50 filtration. After concentration to 2 g/100 ml by vacuum dialysis the fractions obtained were also studied by a gel micro-electrophoresis. Light chains (κ or λ) were identified in IE of concentrated protein peaks obtained by filtration on Sephadex G 200 of whole serum and urine concentrated 100 times by vacuum dialysis.

Vertical starch gel electrophoresis was performed in 0.1 M urea and formic acid buffer at pH 3.0. The purified M-components from serum and urine were reduced by means of β -mercapto-ethanol and alkylated by means of iodoacetamide (16).

Immunologically pure human IgG globulin was used as reference standard for H and L chains after reduction and alkylation. The IgG globulin was isolated from 200 ml human serum by column chromatography on DEAE cellulose (dimensions 1 x 30 cm). The first eluted peak, concentrated to 5 g/100 ml was shown by IE to contain only the slow migrating IgG globulin.

The reagent grade chemicals used were all of highest obtainable purity from British Drug House.

Examination of the hand and foot prints was performed by L. S. Penrose, London, and the identification of κ and λ chains by H. Olsen, Bispebjerg Hospital, Copenhagen.

RESULTS

1 Cytogenetical observations The patient was chromatin negative as determined on Feulgen stained buccal smears. The results of blood cultures (on three separate samples within four months) and on skin cultures at the same time as the second blood culture are shown in Table I.

All cells with 46 chromosomes contained five

Table I Chromosome count distribution on blood and skin cultures from case V H

	Chromosome count distribution					Total
	<45	45	46	47	>47	
Blood I	1	1	111	7	—	25
Blood II	1	—	21	13	—	35
Blood III	—	5	12	3	—	20
Total blood	2	6	49	23	—	80
Skin	—	2	28	—	—	30

small acrocentrics. In nearly all cells the Y was discernible. In one of the 47 chromosome cells the extra chromosome was medium sized submetacentric.

Analysis of cells with 46 chromosomes revealed a normal male karyotype. Cells with 47 chromosomes showed a normal male karyotype and an additional small acrocentric chromosome indistinguishable from the other members of the group 21-22.

2 Dermatoglyphic examination There was no marked indication of mongolism in the lines of the hands or feet. The sum of the maximal at angles was 93° , the mean for control males $85^\circ \pm 15^\circ$ and for mongol males $137 \pm 27^\circ$.

3 Results of protein investigations Fig 2a demonstrates the finding of 1.5 μ l serum applied in IE. By means of an antiserum (80 μ l) from horse against pooled normal human serum the precipitation arc for the IgG globulin in the β 2 area deflects towards the antibody trough. This deflection can be seen more distinctly in immunoelectrophoresis with a rabbit antiserum (80 μ l) strictly specific to the IgG globulin. Furthermore the abnormal arc coalesces with the normal γ globulin arc. The deflection of the IgG globulin in immunoprecipitate indicates an excess in a well

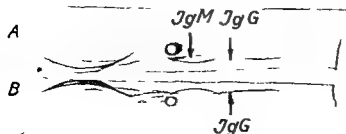


Fig 2a Immunoelectrophoresis of the pathological serum (upper filling hole A) correlated with that of normal serum (lower filling hole B). The electrophoresis is developed with an antiserum against whole human serum (horse antiserum). Two anomalous arcs are seen with pathological pattern: one corresponding to the IgG globulin and one to the IgM globulin.

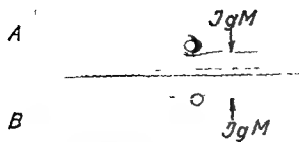


Fig 2b Immuno-electrophoresis of the pathological serum (upper filling hole A) correlated to that of normal serum (lower filling hole B). The electrophoresis is developed with an antiserum against IgM macroglobulin. A double humped arc corresponding to the anomalous IgM globulin is seen. A weak striation phenomenon cathodic to the filling hole is also demonstrated.

defined mobility interval of the corresponding protein antigen of paraprotein character. No Bence Jones protein was noted.

Furthermore in and cathodically to the central filling hole a dense coloured zone can be seen. This is an indication of a high molecular component (molecular weight above 200 000) which can not penetrate through the micelle structure of the gel. Furthermore a weak but distinct precipitate around this zone can be seen. However by treatment of the serum with 0.5% neutralized cysteine a rabbit antiserum specific to IgM will now give rise to the appearance of a distinct precipitate for this immunoglobulin (Fig 2b). This precipitate deflects towards the antibody trough as a sign of a localized excess of an IgM paraprotein in the mobility interval cathodically to the central filling hole.

Agar gel micro-electrophoresis reveals two dis-

tinct M component bands most easily seen in electrophoresis of the dialysed and concentrated (2g/100 ml) supernatant from 1.25 M ammonium sulphate precipitation (Fig 3). Cathodically to the transverse filling trough a distinct dense zone of protein can be seen corresponding to the IgM paraprotein. More cathodically another abnormal M-component appears (IgG paraprotein).

Immunoelectrophoresis of urine (1.5 ml) concentrated 100 times by vacuum dialysis revealed precipitates for albumin, transferrin and an arc situated in the β_2 area close to the antibody trough. This arc develops in IE with an antiserum specific to IgG.

Ammonium sulphate fractionation and agar gel microelectrophoresis of whole serum revealed 80% of the paraprotein components to be precipitated in the interval from 1.25 to 2.20 M ammonium sulphate (pH 6.8).

The abnormal IgG globulin free of IgM was precipitated in the interval of 1.25 to 1.90 M ammonium sulphate. The precipitate obtained in the range of 1.9 to 2.2 M free of IgG was used as a source for isolation of immunologically pure IgM component. Sephadex filtration of this fraction (Sephadex G 200) gave purified IgM component (98% pure state) as the first small sharp peak.

The IgG from the urine was isolated by column chromatography on DEAE cellulose of twenty-four hours concentrated and dialysed urine. Batches of 1 ml dialysed and concentrated urine (4.5 g protein/100 ml) were chromatographed. The IgG fraction was isolated by elution with low ionic strength (2000 to 5000 μ Siemens conductivity).

Immunologically pure human IgG (the isolated IgM paraprotein and the isolated urinary IgG fraction) were reduced and alkylated. After dialysis of the fractions against 8 M urea the reduced and alkylated fractions were subjected to vertical starch gel electrophoresis. Fig 4 demonstrates the



Fig 3 Agar gel microelectrophoresis of pathological serum precipitated in the range from 1.2-1.9 M ammonium sulphate. Two abnormal fractions are seen corresponding to the IgM and IgG globulin.

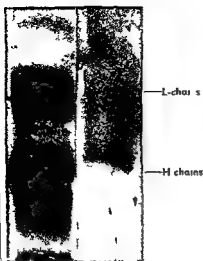


Fig 4 Starch gel electrophoresis of anomalous IgM globulin isolated from serum (to the left) correlated to abnormal component isolated from urine (to the right). The fractions isolated were reduced and alkylated prior to the electrophoresis (see text)

results. The IgM paraprotein reduced and alkylated gave two main bands in the starch gel corresponding to the L and the H chains. Some less well stained bands were apparent. The reduced and alkylated urinary IgG revealed only two protein bands, one with mobility overlapping the faster part of the H band and one corresponding to the L-chains. No other bands could be traced.

Immunoelectrophoresis of concentrated urine and peaks from Sephadex G 200 filtration of serum revealed only the presence of kappa L chains (Fig 5).

DISCUSSION

Cytogenetical discussion

In cases of paraproteinaemia abnormal cell lines with an extra large meta- or submetacentric chromosome have been described in peripheral blood (3, 11, 12, 15, 32).

Castoldi et al (5) found a modal number of 44 chromosomes only in bone marrow cells in a case of multiple myeloma. The karyotype seemed variable. In three cases of the same disease Lewis et al (21) found a mixture of normal and hyperdiploid cells.

Bottura (4) examined five cases of multiple myeloma for chromosome abnormalities. One of these

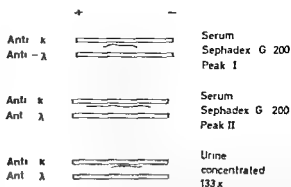


Fig 5 Immunoelectrophoresis with antisera against kappa and lambda chains after fractionation on Sephadex G 200. Peak I contained IgM globulin. Peak II the IgG globulin and the urine IgG globulin (see text).

patients suffered from an α myeloma and chromosome studies on bone marrow cells revealed two lines: one with a normal male karyotype and one with 45 chromosomes. The abnormal cells all lacked a small acrocentric chromosome while the Y chromosome was present.

The karyotype found in a proportion of the patient's blood cells was identical with that described in Down's syndrome (mongolism). The question arises whether this case is one of paraproteinaemia in a patient with a chromosome mosaicism of normal cells and cells with a 21 trisomy. An answer can probably never be given.

The reports on chromosome mosaicism in Down's syndrome are few. Clarke et al (16) found a mosaic in skin cells but normal cells in blood. In some other cases blood cells alone have been studied and chimera-like conditions as found in our patient may thus have been overlooked. All authors found some mongoloid clinical features in their patients. Blank et al (2) found a mosaic in the clinically normal mother of a patient with Down's syndrome.

The present findings did not indicate Down's syndrome and this disease was absent among the relatives. Therefore there may be a connection between the chromosome abnormality and the observed protein abnormality.

The abnormal cell lines cannot be caused by cytotoxic agents or blood transfusion since such treatments were never given.

In the case of an α myeloma described by Bottura (4) a member of the 21-22 group was also involved though lacking in contrast to our case.

Clinical protein chemical and histological discussion

Low levels of vitamin B₁₂ in serum and/or deficiency of folic acid are known in patients with myeloma (20-24, 25) primary macroglobulinaemia (8) and other neoplastic diseases (30). The vitamin B₁₂ deficiency in these cases has been explained by consumption of vitamin B₁₂ by the growing malignant cell populations. Furthermore transport of vitamin B₁₂ due to abnormal or lacking plasma proteins may give rise to megaloblastic anaemia (17) but these patients do not respond to vitamin B₁₂ therapy.

In the present case serum bilirubin and serum iron showed normal values but otherwise the clinical findings and laboratory data suggested a typical pernicious anaemia.

The chemical investigations indicate the presence in serum of two M components as previously described by Drivsholm and Clausen (9) and Kjeldsen and Asfeldt (19). The case story is difficult to evaluate as primary macroglobulinaemia and multiple myeloma exhibit a number of similar signs. A precise diagnostic classification of some cases of M components is difficult or impossible (28, 33).

The use of electrophoresis as a routine laboratory procedure unveils cases with M components which initially do not represent myeloma or primary macroglobulinaemia. Some of these cases later develop clinically typical myeloma or macroglobulinaemia (27). There remain however a number of cases with M-components which fail to develop an identifiable clinical pattern (33, 34). Usually these so-called monoclonal gammopathias are an IgG type protein abnormality although IgA and IgM exist.

In primary macroglobulinaemia the Bence Jones protein excreted in the urine belongs to the L-chain type which also is found in most patients with multiple myeloma (10, 16). In the present case the gamma globulin component present in the urine seems similarly to be of the kappa-chain type which also was found in the serum para-proteins.

A correlation between the immuno-electrophoretic findings in serum and urine and the morphological characteristics of the cells responsible for their production has been reported (9, 29, 33). The flame cells and "thesaurocytes" are thus ex-

clusively found in IgA myelomas. The formation of IgM protein usually takes place in the lymphoid reticulum cells (1).

The present case seems to be another one of the still more frequent obscure clinical pictures combined with the presence of M-components in serum and/or urine. Instead of attempting a precise clinical classification it may be more satisfying to classify these patients according to their protein chemical abnormality.

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LACTASE DEFICIENCY AND A LACTOSE FREE DIET IN PATIENTS WITH UNSPECIFIC ABDOMINAL COMPLAINTS

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Abstract Jejunal lactase activities have been determined in 41 patients who had consulted general practitioners on account of unspecific abdominal complaints. Twelve of these patients had low jejunal lactase activity and they all had selective lactase deficiency.

A lactose free diet for two weeks had a beneficial effect on all the patients with low lactase activity and the effect was not temporary as could be noted on inquiry about six months later.

It is remarkable that only two of the twelve patients with low lactase activity had a history of milk intolerance.

Determination of small intestinal lactase activity or a peroral lactose tolerance test is recommended as a routine test for milk-drinking patients with unspecific abdominal complaints.

According to a recent interview survey abdominal symptoms and diseases are the third most common group of chronic diseases in the adult population in Finland and about 15% of those interviewed suffered from abdominal symptoms without any demonstrated organic disease (14). This problem may be worldwide for in the USA digestive diseases ranked fourth as a cause of total economic cost of illness grouped by major diagnostic categories (2) and they were the second most common cause of disability among a selected group of employees (4). On the other hand it is well known that small intestinal lactase deficiency is rather common and causes diarrhoea, abdominal fullness and meteorism after ingestion of lactose-containing foods almost the same symptoms as are reported by patients with "unspecific abdominal complaints" and with nonspecific gastroenteritis and diarrhoea. In accordance with this it has been shown that many hospital patients with functional diarrhoea (12) and irritable colon syndrome (15) had lactase deficiency and their symptoms diminished on a lactose free diet.

Lactose intolerance is an important problem in countries where milk consumption is high. Milk consumption in Finland during 1965 was 290 kg per head (11), one of the highest in the whole world and about twice as high as in the U.S.A. (6).

It therefore seemed especially necessary to clarify

1) the frequency of lactase deficiency in patients with unspecific abdominal complaints visiting general practitioners

2) the effect of a lactose free diet on the symptoms of these patients

This study deals with these problems

SELECTION OF PATIENTS

From the consulting rooms of the two doctors taking part in the investigation were remitted those patients with abdominal complaints who in X-ray and laboratory examinations had been verified not to have any organic disease of the digestive organs and who in addition to this also fulfilled the following criteria

1) patients had at least one of the following symptoms: abdominal fullness, feeling of nausea, unidentified abdominal distress, meteorism, loose stools, watery diarrhoea

2) patients had the symptoms on average at least once a week and had had them at least more than one month before consulting the doctor

3) patients sought medical aid because of abdominal distress or spontaneously mentioned the symptoms during the examination or treatment of some other disease

4) no other medicament influencing abdominal symptoms than Enzynorm® tablets were necessary during the experiment

MATERIAL

Altogether 43 patients who fulfilled the above criteria visited the two general practitioners taking part in the investigation during the period January to May 1968. Two refused small intestinal biopsy and were not included in the study. Small intestinal specimens were taken from 41 patients, 32 of whom were women, 27 to 62 years of age (mean 47.5) and 9 men, 28 to 67 (mean 41.5 years).

METHODS

Design of diet experiment

During the study the patients were

- one week on a lactose free diet (week I)
one week on their customary diet (week II)
one week on a lactose free diet (week III)

During the lactose free diet foods containing milk and lactose were forbidden. However cheese, butter and sour milk products were allowed because in cheese and butter lactose is only presented in minimal amounts if at all and in sour milk products lactose is already partly hydrolysed (9) and probably lactose splitting bacteria continue their action in the stomach and small intestine where an optimal temperature for their function prevails.

1 The customary diet did not differ from the food habits of the patients.

The effect of diet was estimated by patients weekly comparing subjective abdominal symptoms and consistency and frequency of stools to the corresponding ones of the previous week. In this way the effect of a short term lactose free diet was noted. The patient and the treating doctor were not informed of the results of lactase determination until the diet experiment was over.

Small intestinal biopsies

Small intestinal biopsies were taken from the jejunum immediately distally of the ligament of Treitz with Bults or Brandborg Rubin's biopsy instrument. The specimens were cut in half and one half was fixed immediately in 10% neutral formalin stained in haematoxylin eosin and examined under the light microscope. The specimens were classified as normal if four normal villi were found adjacent to each other. The amount of round cell infiltration in the *lamina propria* of the small intestinal mucosa was roughly estimated. The other half was stored at -20°C until determination of disaccharidase activities according to a modification (10) of the original method of Dahlqvist (5). The disaccharidase activity is expressed as units/g mucosa wet weight. The lowest limit of normal lactase activity with the method used is 1.5 units and that of the normal lactase to sucrose ratio is 0.3. The limits are estimated on the basis of 89 consecutive struc-

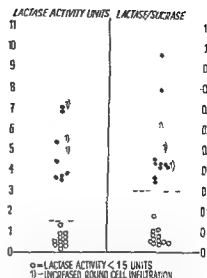


Fig 1 Distribution of jejunal lactase activity and lactase to sucrase ratio in the patients investigated

turally normal biopsies the analysis of which according to McMichael (12) is presented elsewhere (7)

Later inquiry

In October 1968 six months or so after the diet experiment a follow up inquiry was made. The patients were asked whether they had abdominal complaints and whether they had continued with the lactose free diet. In this way it was possible to notice the effect of the long term lactose free diet on some of the patients investigated. In the case of symptomless patients still on the lactose free diet, the patient was asked to drink a glass of milk twice a day during meals for three consecutive days. The harmful effects of milk were then to be reported. In the later inquiry information was received from 39 patients.

RESULTS

Small intestinal biopsies

None of the patients had villous changes but three had increased round cell infiltration in the lamina propria of the small intestine. Of altogether 41 patients examined 12 had low jejunal lactase activity (less than 1.5 units) and all of these but none of the others had a low lactase to sucrose ratio (less than 0.3) as shown in Fig. 1.

Subjective symptoms

Patients with low lactase activity did not differ from the others in regard to the nature and duration of the abdominal symptoms as shown in Table I.

Table I Abdominal symptoms and their duration in the patients investigated

	Normal lactase activity				Low lactase activity			
	Duration of symptoms		No of pats	Symptoms of pats	Duration of symptoms		No of pats	Symptoms of pats
	< 12 mo	> 12 mo			< 12 mo	> 12 mo		
Abdominal fullness	5	24	29	100	2	9	11	92
Meteorism	4	18	22	76	3	8	10	83
Nausea	5	11	16	53	1	5	6	50
Unidentified abdominal complaint	3	10	13	45	1	4	5	42
Loose stools	2	9	11	38	2	3	5	4
Watery diarrhoea	3	1	4	14	1	2	3	25
Obstipation	1	4	5	17	—	1	1	8

Three of those investigated had a history of milk intolerance and two of them had low lactase activity

Short term lactose free diet

The short term lactose free diet had a beneficial effect on all the patients with low lactase activity and on some of the patients with normal lactase activity as shown in Table II. The mean lactase activity of the patients with normal lactase activity noting a beneficial effect of the short term lactose free diet was 4.1 and that of the others with normal lactase activity was 5.0 (Fig. 2). The difference is not statistically significant.

The mean age of the patients with a low lactase activity was nearly the same as that of patients with normal lactase activity: 47.5 and 46 years respectively.

Later inquiry

According to Table III all the patients with low lactase activity were without symptoms at the later inquiry and they had all continued a lactose free diet for more than six months after the beginning of the experiment. After drinking two glasses of milk daily for three consecutive days eight of them reported abdominal complaints while three remained symptom free. Of the patients with normal lactase activity only eight were symptom free at the time of later inquiry. Of these symptom free patients two had been subjected to gall bladder operation during the time after the short term lactose free diet and before the later inquiry and three others attributed their freedom from symptoms to the regular use of Enzy-norm[®] tablets.

Five of the 28 patients with normal lactase activity still continued on a lactose free diet; only one

Table II The effect of the lactose free diet on the abdominal symptoms in the patients investigated

	Abdominal symptoms aggravated	Abdominal symptoms unchanged	Abdominal symptoms lessened	Abdominal symptoms totally disappeared	No of pats
<i>1st week lactose free diet</i>					
Normal lactase activity	1	19	—	—	29
Low lactase activity	—	2	8	2	1
<i>2nd week lactose free diet</i>					
Normal lactase activity	14	9	3	1	27 ^a
Low lactase activity	10	—	—	2 ^b	12
<i>3rd week lactose free diet</i>					
Normal lactase activity	3	10	7	2	21 ^a
Low lactase activity	—	—	6	4-2 ^b	12

^a Some of patients abandoned diet experiment

^b Patients refused to drink milk

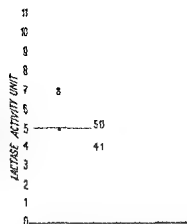


Fig. 2 Distribution of jejunal lactase activity in patients with normal lactase activity and the effect of a short term lactose free diet. Symbols: ○ = short term lactose free diet had a beneficial effect; — = the mean of these patients; ● = short term lactose free diet did not have a beneficial effect.

of these five had no symptoms and she had abdominal complaints when she started to drink milk again.

The milk habits of the patients were not recorded but six patients with low lactase activity stated that they had drunk unusually large amounts of milk during the period before the experiment leaving that milk would be beneficial for their abdominal symptoms.

CASE REPORTS

Two case reports are given which illustrate the good effect of the lactose free diet.

Case 1

A 34 year-old engineer who for more than four years had had upper abdominal pain, nausea and diarrhoea. He had a history of milk intolerance. In 1966 X-ray examination of the stomach revealed deformatio bulbi. Anticholinergic medication did not help. In 1967 the patient visited the doctor three times because of abdominal complaints. In 1968 X-ray examination revealed mild gastritis, the gall bladder was roentgenologically normal as was the result of aspiration of duodenal contents after secretin stimulation. The patient's abdominal complaints disappeared during the short period on a lactose free diet and on later inquiry the patient was without symptoms and had continued with the lactose free diet. The patient developed marked abdominal symptoms on starting to drink milk again. Jejunal lactase activity was low.

Case 2

A 41 year-old foreman's wife who has consulted many doctors several times during 5-6 years because of abdominal pain, nausea and diarrhoea. During these years the stomach had been examined roentgenologically three times and the gall bladder once every time with normal result. The patient had been told to drink milk to relieve the abdominal complaints and she has been treated as a neurotic with tranquilizers. The patient's abdominal complaints disappeared during the short term lactose free diet and at the time of the later inquiry she was without symptoms and had continued with the lactose free diet. The patient had no recurrence of abdominal complaints on drinking two glasses of milk daily during three consecutive days as requested. Jejunal lactase activity was low.

DISCUSSION

According to this investigation the frequency of lactase deficiency was 29% in patients suffering from unspecific abdominal complaints and representing the general practitioners' patient material.

Table III The results of the later inquiry to 39 of the patients investigated

	Abdominal complaints		Lactose free diet		Effect of milk		No. of patients
	Present	No	Continued	Abandoned	Causes abdominal symptoms	Does not cause abdominal symptoms	
Low lactase activity	—	11	11	—	8	3	11
Normal lactase activity							
Short term lactose free diet beneficial	5	4 ^a	5	4	1	—	9
Short term lactose free diet not beneficial	15	4 ^b	—	19	—	—	19

^a Two patients had gall bladder operation during time between short term lactose free diet and later inquiry.

^b Three patients attributed freedom from symptoms to regular use of Enzymorm[®] tablets.

terial This generality corresponds to the lactase deficiency verified in hospital patients suffering from functional diarrhoea (13) and irritable colon syndrome (15) The frequency of lactase deficiency has been verified to vary considerably as a result of racial and genetic factors (1 3 12) which has to be taken into account in comparisons of investigations carried out in different countries

The significance of the frequency of this verified lactase deficiency still remains somewhat obscure because of lack of information on the general prevalence of lactase deficiency Some kind of standard of comparison is supplied by investigations carried out in Finland on selected hospital cases and on servicemen (8) According to this investigation 17% of the hospital patients averaging 43 years and 18% of the servicemen averaging 20 years were verified to have lactose intolerance i.e. lactase deficiency Among the hospital cases 48% of the patients with a history of milk intolerance 21% of those suffering from repeatedly occurring abdominal complaints but without a history of milk intolerance and 7% of those with no abdominal complaints or history of milk intolerance had lactose intolerance In 15% of the servicemen who did not have abdominal complaints or a history of milk intolerance lactose intolerance was correspondingly verified

These facts suggest that there is no further increase in the occurrence of lactase deficiency once adult age is reached but that the deficiency becomes clinically manifest i.e. lactose intolerance appears at different ages especially in adult life This hypothesis is supported by the clear separation of the lactase population and lactase to sucrose ratios into two groups without any intermediate forms as shown in Fig 1 and noted earlier (7 12) This being the case it is further very possible that when during the manifestation of lactose intolerance a patient with lactase deficiency continues to consume milk without noticing its harmful effects he becomes one of the patients with unspecific abdominal complaints This would also explain the fact that in our investigation only two out of 12 patients with unspecific abdominal complaints and lactase deficiency had a history of milk intolerance

The present investigation shows that it is important to diagnose lactase deficiency in patients with "unspecific abdominal complaints" for this deficiency is common in them and the effect of a

lactose free diet is indisputable The effect is not transient as was proved by the results of the later inquiry This also showed that after the symptoms had subsided some of the patients could take a small daily portion of lactose without developing symptoms The diagnosis of lactase deficiency in patients with unspecific abdominal complaints cannot be made on the basis of the case history or subjective symptoms because a history of milk intolerance is seldom associated with lactase deficiency by these patients and the symptoms are the same in patients with normal lactase activity Nor on the basis of the effect of a short term lactose free diet does it seem possible to diagnose lactase deficiency with certainty However failure of the abdominal complaints of the patient to decrease during a lactose free diet strongly suggests a normal lactase activity The diagnosis can be confirmed by determination of jejunal lactase activity or by a lactose tolerance test Because a satisfactory correlation exists between jejunal lactase activity and the result of a peroral lactose tolerance test the latter can be used as a routine test for milk-drinking patients with unspecific abdominal complaints

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HYPERPYREXIA IN ASSOCIATION WITH ADMINISTRATION OF L ALPHA METHYLDOPA

A Report of Two Cases

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Abstract Two cases of hyperpyrexia associated with administration of L alpha methyl dopa are presented. Febrile reaction has been reported to both D and L isomers of methyl dopa but true hyperpyrexia has occurred only with the L isomer. Previous attempts to obtain proof of an allergic reaction as a cause of hyperpyrexia have failed. In vitro studies were made of the leukocytes of one of the patients sensitized by administration of L alpha methyl dopa, which was also used as an antigenic agent in the experiment. The results suggest the possibility of toxic leukocytolysis as the causative mechanism underlying the hyperpyrexia.

Alpha methyl dopa (alpha methyl 3,4-dihydroxy L phenylalanine) a decarboxylase inhibitor has a well-established position in antihypertensive therapy to-day. The drug is thought to decrease the tissue stores of catecholamines. Although side effects are frequent they are usually mild and do not as a rule necessitate withdrawal of the drug. The adverse reactions to methyl dopa as reported in medical literature have already been extensively reviewed elsewhere (9). The major side effects mentioned in order of frequency and severity are liver toxicity, fluid retention, angina pectoris and febrile reactions. Febrile reactions have usually been reported in a frequency of 1 to 3% although as many as 6% have been encountered (5, 6, 7, 11). Febrile reactions have been reported to both D and L isomers although true hyperpyrexia has only been described in connection with the administration of the L isomer (8). The febrile reactions have usually been mild and the temperature has not exceeded 40°C. To our knowledge only one case of hyperpyrexia has so far been published (8). In the two cases to be described below there were hyperpyretic reactions in connection with the administration of the L isomer of

methyl dopa. This type of reaction has been regarded as allergic (8). In order to test this hypothesis in vitro studies of the leukocytes of case 2 were carried out.

CASE REPORTS

Case 1

The patient was a 4-year-old male agriculturalist. A transiently elevated blood pressure had been recorded in 1961 in connection with an operation on an intervertebral disc prolapse of the lumbar spine. In June 1966 the patient was admitted to hospital because of intense headache, impaired vision and elevated blood pressure (240/160 mm Hg). Examinations revealed bilateral astigmatism and grade IV hypertensive retinal changes. The serum creatinine was slightly elevated 1.5 mg/100 ml. Chest X-ray showed dilatation of the left ventricle and corresponding ECG changes were recorded (left axis deviation and left side strain). Renal biopsy showed mild arteriolar hypertensive changes and some mild capsular adhesions in the glomeruli. The patient received 750 mg L alpha methyl dopa divided into three doses from July 7, 1966 and in addition 40 mg furosemide daily. The patient was discharged on Aug. 8, 1966 with this medication; the blood pressure having dropped to 230/120 mm Hg with marked subjective improvement. On Aug. 5, 1966 he had a temperature of 39.1°C, haematuria and pyuria without bacteriuria and blood pressure 190/135 mm Hg. The medication was stopped. On Aug. 10, 1966 methyl dopa and furosemide were reconstituted in the same dose and the temperature rose within two days to 40°C. The patient was readmitted on Aug. 13, 1966. No signs of infection could be found. Haemoglobin, however, had fallen from 12.0 (Aug. 10, 1966) to 9.6 g/100 ml (Aug. 19, 1966) without any signs of bleeding or haemolysis, normal bilirubin, reticulocytes 2%, elevated haptoglobin level, direct Coombs test negative. Furthermore there was a leukocytosis 11,400/mm³ and eosinophilia 35%. Methyl dopa was once more withdrawn and the temperature returned to normal within a day. In order to test the causative relationship between methyl dopa and the febrile reactions the drug was re-

instituted one and a half weeks later in a dose of 500 mg a day and the febrile reaction reappeared within three days. It thus seemed highly probable that the hyperpyrexia was a side effect of methyldopa. The patient was thereafter treated with guanethidine sulphate to which furosemide and later hydralazine were added. His blood pressure responded very poorly, the renal function gradually deteriorating. On Aug. 9, 1967, he was readmitted to hospital because of his greatly impaired general condition and a rise in the serum creatinine level from 4.4 to 11.0 mg/100 ml within a week. The patient died on Aug. 15, 1967, from acute pulmonary oedema as a complication of uraemia and congestive heart failure. Autopsy showed renal changes typical of malignant hypertension with arterial necrosis. No primary myocardial or pulmonary disease could be detected.

Case 2

A 52-year-old woman was admitted to hospital in 1957 because of benign essential hypertension of 10 years standing as well as enlargement of the heart. The initial systolic blood pressure had been 330 mm Hg. The patient however became symptom free within some months on oral antihypertensive therapy after which she abstained from further medical supervision. About ten years later a routine public health chest X-ray revealed cardiac enlargement. On Feb. 27, 1967, two months later she had a nocturnal attack of dyspnoea. Her blood pressure was 270/140 mm at that time and she received reserpine 125 mg, chlorthalidate 250 mg and digoxin 0.25 mg once daily. On July 6, 1967, she consulted a physician. The following findings were made: blood pressure 300/140 mm, a regular pulse rate of 100/min, an accentuated second sound and a grade II systolic murmur over the aortic valve, no enlargement of the liver or peripheral pitting oedema, grade I hypertensive retinal changes, varicose veins of both legs, weight 85 kg. Chest X-ray on July 13, 1967, no parenchymal lung process or signs of cardiac congestion, elongation of the aorta, all round cardiac enlargement with total heart volume of 1200 ml, equalling 640 ml/body surface sqm. Laboratory findings: serum creatinine 0.76 mg/100 ml, Na 141 mEq/l, K 4.3 mEq/l, ESR 27 mm/1 h, haemoglobin 13.3 g/100 ml, leukocytes 7200/mm, urinalysis showed no protein or glucose, pH 5.0, sp weight 1014 and in the sediment red cells 4/Hpf, white cells 60/Hpf and Gram negative rods. Therapy prescribed on July 6, 1967: digoxin 0.25 mg, chlorthalidate 500 mg, 1 alpha-methyldopa 750 mg divided into four doses. On July 19, 1967, the patient had diarrhoea and temperature at 40°C. After this she had several hyperpyretic reactions usually up to 40°C recurring irregularly in the evenings. The gastrointestinal symptoms rapidly subsided with diphenhydramate chloride and atropine sulphate. Because of the febrile reactions the patient was referred to the hospital out-patient department on July 25, 1967, where the findings were: blood pressure 230/130 mm Hg, weight 84.5 kg, no oedema. She was admitted to the hospital ward on July 26, 1967. The methyldopa treatment was reinstituted (750 mg/daily) and there was an immediate rise in temperature to 40.1°C. The drug was withdrawn on the following day and the temperature fell to 38.4°C, having returned to normal by July 31, 1967.

The same medication was repeated on Aug. 8 and Aug. 10, 1967, the patient reacting with temperatures of 39.4°C and 38.8°C respectively. The hypotensive effect was satisfactory, the blood pressure falling to 145/100 mm Hg without other side effects apart from hyperpyrexia accompanied by leukocytosis (10,400/mm³) with eosinophilia of 1000 cells/mm³ (July 28, 1967).

On July 27, 1967, ESR was 62 mm/1 h. It was concluded that the hyperpyretic reactions were associated with the administration of methyldopa. With a regimen of Serpasil® 0.1 mg three times daily and 500 mg of chlorothiazide with potassium substitution the patient's hypertension was sufficiently controlled (180/100 mm Hg). Digoxin was continued as before.

LEUKOCYTE TESTS METHOD AND RESULTS

An attempt to clarify the mechanism causing the hyperpyretic reaction was made by studying the patient's (case 2) leukocytes *in vitro*. Strong antigens are known to cause rapid destruction of leukocytes *in vitro* in the simultaneous presence of both antigen and serum from sensitized individuals (1, 3). Our intention was to record the *in vitro* reaction of the leukocytes from the patient sensitized by administration of 1 alpha-methyldopa which was also used as an antigenic agent in the experiment. The white cell reaction was observed before, during and after the clinical hyperpyretic reaction. Leukocytolysis is regarded as a phenomenon preceding febrile reactions (4, 10).

The leukocytes were separated from the blood cells according to the method of Boyle and Tulikainen (2). 1 alpha-methyldopa (Dopamet® A/S, Dumer, Denmark) 2.0 mg was dissolved in 10 ml saline and serial dilutions were prepared from the stock solution. One drop of the solution obtained was put in a specially constructed well slide and the solution allowed to dry. The suspension of the patient's leukocytes and serum was pipetted to the dry antigen. The reaction of the leukocytes was recorded half an hour later under the microscope before and after staining with methylene blue.

The results of the leukocyte tests are presented in Table I. It shows that the strongest solutions of methyldopa had a similar leukotoxic effect upon the white cells of the patient as upon those of the control. Practically all cells were destroyed. If the concentration of methyldopa was diluted the leukocytolytic reaction became weaker and agglutination occurred. This reaction too disappeared with the weakest solutions. There was only a quantitative difference between the leukocyte reactions of the patient and the control, the leukocytes of

Table I Leukocyte tests *in vitro* with 1 alpha methyl dopa

Dilution	Cell agglutination	Devitalized cells ()
<i>The patient a white cell reaction before hyperpyrexia</i>		
1	None	100
1 2	None	100
1 4	None	100
1 8	None	90
1 16	None	50
1 32	Occurred	III
1 64	Occurred	50
<i>The patient a white cell reaction during hyperpyrexia</i>		
1	None	100
1 2	None	90
1 4	Occurred	50
1 8	Occurred	20
1 16	Occurred	5
1 32	Occurred	5
1 64	None	5
<i>The patient a white cell reaction four days after normalization of temperature</i>		
1	None	100
1 2	None	100
1 4	None	95
1 8	None	50
1 16	Occurred	50
1 32	Occurred	30
1 64	Occurred	30
<i>Control subject a white cell reaction</i>		
1	None	100
1 2	None	90
1 4	Occurred	50
1 8	Occurred	15
1 16	Occurred	5
1 32	Occurred	5
1 64	None	5

the sensitized patient having only a lower tolerance to the antigenic agent. During the hyperpyretic reaction the patient's leukocytes however behaved like those of the control. This is interpreted as evidence that the patient's sensitized white cells had undergone lysis *in vivo* immediately prior to the febrile peaks and that the sample taken during the febrile phase mainly contained still unsensitized leukocytes. Four days later the "normal tolerance" was lost i.e. the reaction that of the sensitized pre febrile state.

COMMENTS

From the data presented above the suggestion can be made that hyperpyretic reactions are associated

with lysis of sensitized leukocytes. Because the reaction *in vitro* gradually increases with the strength of the solution we have regarded it as a toxic rather than an allergic reaction. Previous attempts to obtain proof of an allergic reaction as a cause of hyperpyrexia have failed. Montegriffo (8) performed a patch test and gave intradermal injections of methyl dopa at increasing strengths (0.1-1.0%) of the L isomer but the results were negative as was the Prausnitz-Kustner reaction. He further attempted to show the presence of circulating antibodies by using simple flocculation and gel diffusion precipitation techniques. No precipitations were demonstrated at any time however. In spite of the negative tests Montegriffo (8) regards the hyperpyrexia observed in his cases as an allergic hypersensitivity reaction mediated by an antigen-antibody reaction although circulating antibodies could not be detected. He stresses however that these are often extremely difficult to demonstrate and that the skin tests rarely detect drug sensitivity. In spite of these negative findings Montegriffo is inclined to regard the hyperpyretic reaction as an allergic one mainly because of the mechanism behind any kind of drug sensitivity and because untoward reactions of this type have usually been regarded as allergic.

Our findings correspond to those of Montegriffo i.e. they give no objective evidence of an allergic reaction. However they suggest the possibility of toxic leukocytolysis as the causative mechanism underlying the hyperpyrexia.

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IN VITRO DETECTION OF TUBERCULIN HYPERSENSITIVITY IN MAN

Specific Migration Inhibition of White Blood Cells from Tuberculin positive Persons

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Abstract The influence of purified protein derivative of tuberculin (PPD) upon the in vitro migration of peripheral leucocytes from persons with positive and negative intracutaneous tuberculin reactions has been investigated. An inhibition is seen with leucocytes from Mantoux positive persons whereas no inhibition is seen with leucocytes from Mantoux negative persons. The migration indices are well correlated to the degree of the intracutaneous reactions in mm.

Rich and Lewis (8, 9) were the first to prove that cellular (delayed type) hypersensitivity in experimental animals can be demonstrated in vitro by examining the migration of immunocompetent cells. The migration is inhibited specifically by antigen to which the animals exhibit a cellular hypersensitivity.

Numerous animal experiments carried out more recently have shown that the inhibition of migration by antigen is a good parameter in vitro for cellular hypersensitivity. Apart from tuberculin a number of other antigens have been employed.

Only few studies of tuberculin hypersensitivity in man are available assessed on the basis of inhibition of cell migration by tuberculin.

It was a characteristic of the early studies that inhibition of migration was demonstrated in cases of active tuberculosis whereas on rare occasions only inhibition was found in non tuberculous persons with a positive cutaneous tuberculin reaction (6, 7). In the cases where inhibition was demonstrated it was not related to the intensity of the intracutaneous tuberculin reaction.

Employing the capillary tube technique described by George and Vaughan (5) and modified by David et al (3) Thor (13, 14) was recently able to demonstrate inhibition of cell migration in

non tuberculous persons with a positive intracutaneous tuberculin reaction. However he did not mention any possible relationship between the degree of the inhibition of migration and the intensity of the intracutaneous tuberculin reaction.

In these studies either human lymph node cells cultivated for three days before application in migration cultures were used (13) or peritoneal exudate cells from guinea pigs to which were added supernatants from cultures of human lymphocytes from the peripheral blood incubated for 24 hours with tuberculin (14).

The method is found to be specific and very sensitive but complicated and time-consuming.

Spørg and Bendixen (10) showed that the capillary tube technique using human peripheral leucocytes is well suited for the demonstration of cellular hypersensitivity. Furthermore in a study of the brucella hypersensitivity it was proved that the degree of the inhibition of cell migration is related to the intensity of the intracutaneous reaction (11).

Compared with the method employed by Thor there are the advantages that the above technique does not require much time, no experimental animals are needed and the test can be carried out using peripheral blood.

The object of the present study was to examine whether the method is suited also for the examination of tuberculin hypersensitivity.

MATERIAL AND METHODS

The tuberculin positive group comprised 38 persons with a positive intracutaneous tuberculin reaction. Thirty-two had a positive Mantoux I, six had a positive Mantoux II.

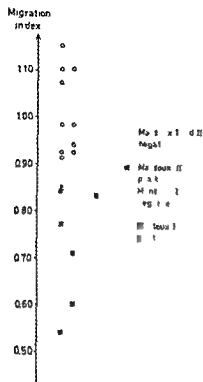


Fig 1 Migration indices of Mantoux positive and Mantoux negative persons

a negative Mantoux I. None of the persons presented signs of active tuberculous infection. Some of them had a BCG vaccination, others were spontaneously tuberculin positive.

The tuberculin negative group comprised 12 persons in whom both Mantoux I and II tests were negative.

Patients with diseases which might influence the cellular hypersensitivity and patients being treated with glucocorticoids or cytostatics were not included in the study.

The leucocyte migration test (LMT) was carried out employing the method described in further detail by Spborg and Bendixen (10). Leucocytes from peripheral blood were washed thoroughly and aspirated into capillary tubes which were placed in 1 ml culture chambers containing a culture medium. To half of the culture chambers 100 μ l of a tuberculin solution were added containing 100 μ g of purified protein derivative (PPD) (placed at our disposal by M. Magnusson, the Tuberculin Department of the State Serum Institute, Copenhagen). To the other half of the chambers were added 100 μ l of the phosphate-buffered solution which is used as a solvent for PPD.

The cell migration areas after 24 hours were measured. The average area in antigen-containing cultures divided by the average area in control cultures is termed the migration index (MI). MI is a measure of the antigen-dependent inhibition. The stronger the inhibition, the lower the MI.

The Mantoux tests were carried out by intracutaneous

injection of 0.1 ml of tuberculin solution. The solution used in Mantoux I contains 1 TU = 1/50 000 mg PPD per 0.1 ml. The solution used in Mantoux II contains 10 TU = 1/5000 mg PPD per 0.1 ml. The skin reaction is read after 72 hours. The reaction is recorded as positive when the induration is 5 mm or more.

LMT was always carried out before examination of the intracutaneous tuberculin reaction.

RESULTS

Fig 1 shows the distribution of the migration indices in 38 persons with positive intracutaneous tuberculin reaction (32 positive after Mantoux I, six positive after Mantoux II but negative after Mantoux I) and 12 persons with negative cutaneous tuberculin reaction after both Mantoux I and II.

The mean value of the MI for the tuberculin positive persons is 0.74 with the standard deviation (s.d.) 0.13.

The mean value for the tuberculin negative persons is 1.01 with s.d. 0.08. The two groups are significantly different ($p < 0.001$).

The lower limit for the normal range of the tuberculin negative persons is 0.85 (mean value -2 s.d.). As appears from the figure there is some overlapping between the two groups so that the MI for some of the tuberculin positive is in the lower half of the tuberculin negative normal range.

Fig 2 shows the relationship between the size in mm of Mantoux I and the MI in both tuber-

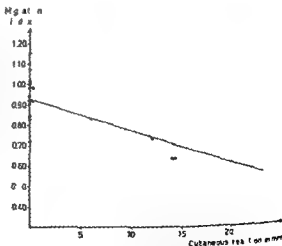


Fig 2 Thirty-eight Mantoux positive and twelve Mantoux negative persons. Correlation of migration index and intracutaneous reaction.

culin positive and tuberculin negative persons. There is a good correlation between the intracutaneous reaction and the MI ($r = -0.61$ $p < 0.001$).

DISCUSSION

The present results seem to indicate that a tuberculin-dependent inhibition of cell migration of peripheral leucocytes is a good parameter *in vitro* for tuberculin hypersensitivity assessed from the intracutaneous tuberculin reaction. However, no inhibition was observed in about 15% of the tuberculin positive persons, which seems to indicate that the sensitivity of the test is not quite satisfactory. On the other hand, in none of the tuberculin negative persons did the MI fall below 0.91. An MI below 0.85 (lower calculated limit of tuberculin negative persons) therefore will show with certainty that the person concerned is tuberculin positive. On the other hand, an MI above 0.85 can be seen in both tuberculin positive and tuberculin negative persons.

In the present study an antigen concentration of 100 μg of PPD per ml was used. It is possible that a better differentiation between tuberculin negative and tuberculin positive persons could be obtained by increasing the tuberculin concentration, but this would involve the risk of a non-specific toxic effect of the tuberculin. On the other hand, the antigen concentration employed is strong enough to ensure good agreement with the conditions *in vivo*, as proved by the positive correlation between the intracutaneous test measured in mm and the inhibition of cell migration.

In the early *in vitro* studies of human tuberculin hypersensitivity (2, 6, 7) tuberculin concentrations of the same order of magnitude were employed as those used in the present study. Notwithstanding a concentration of 100 microgrammes of tuberculin per ml, O'Neill and Favour (7) were not able to demonstrate any inhibition of the leucocyte migration in 11 out of 21 patients with tuberculosis. Hall and Scherago (6) with a similar tuberculin concentration found that the cell migration in patients with active tuberculosis was distinctly inhibited in only 67% of the cases. Patients with inactive tuberculosis and healthy persons with positive intracutaneous tuberculin reaction showed inhibition in only 21% and 9% of the cases respectively.

Thor (13, 14) used a tuberculin concentration of 10 μg per ml. This was the case both in the experiments when he used lymph node cells and in the experiments when he used peritoneal exudate cells from guinea pigs to which were added supernatants from cultures of human peripheral lymphocytes cultivated for 24 hours with PPD. Employing both experimental methods, migration indices of the order of magnitude 0.20–0.30 were obtained.

The difference between the necessary tuberculin concentration in the present study and in Thor's studies and the difference in the degree of the inhibition of migration are presumably caused by the employment of different cell systems.

The influence of the composition of the cell population was first demonstrated by David et al. (4) who showed that the migration of sensitive lymphocytes separated from lymph node cells was not inhibited by addition of the specific antigen, whereas this produced a pronounced inhibition when added to a mixture of sensitive lymphocytes and macrophages. Bloom and Bennet (1) showed that the migration of macrophages separated from peritoneal exudate cells from hypersensitive animals was also not inhibited by the antigen concerned. The conclusion of these studies was that two types of cell were required for producing inhibition of cell migration by antigen. One cell type should be sensitive lymphocytes, the other macrophages which need not of necessity originate from a hypersensitive organism.

In experiments with peripheral human leucocytes, Spborg (12) showed that sensitive lymphocytes separated from the blood could also not be inhibited by addition of antigen, but that the presence of granulocytes and monocytes was necessary for the inhibition of migration.

In the first studies carried out by Thor (13) the approximate composition of the cell population after cultivation of the lymph node cells for three days was macrophages 25% and lymphocyte-like cells 65%, the remaining cells being non-classifiable. In his most recent studies (14) the cell population is peritoneal exudate cells from guinea pigs, mainly macrophages, but also some lymphocyte-like cells.

It remains to be clarified how macrophages and a mixture of granulocytes and monocytes respectively contribute to an inhibition of the migration of the entire cell population. The difference be-

tween the results obtained by Thor and by us however might be explained by the fact that macrophages are more effective than a mixture of granulocytes and monocytes in mediating the inhibition of migration of the total cell population

ACKNOWLEDGEMENTS

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CENTRAL HEMODYNAMIC EFFECTS OF TERODILINE IN PATIENTS WITH CORONARY HEART DISEASE

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Abstract The hemodynamic response to terodiline has been tested on ten patients with severe coronary heart disease. The drug had no significant effects on the central hemodynamics at rest. During exercise, however, terodiline reduced the pulmonary capillary venous pressure at an increased or unchanged calculated external cardiac work in eight of the patients. No pain or reduced pain during exercise was noted by four of these patients.

Terodiline or Bior® (1-methyl-3,3-diphenyl-1-N-tert-butyl-propylamine hydrochloride) possesses a slight local anesthetic effect and also has anticholinergic, spasmolytic and antihistaminic properties. It has no effect on adrenergic α or β receptors. In animal experiments using different pharmacological standard techniques, terodiline produces marked increases in coronary blood flow through relaxation of smooth muscle cells in the vascular wall, resulting in decreased coronary vascular resistance (4) (Terodiline (Bior®) from AB Recip, Stockholm).

It has therefore been suggested that this drug could be used in the treatment of coronary insufficiency (angina pectoris) (5).

In some clinical trials it has been demonstrated that terodiline has beneficial effects on patients with angina pectoris due to coronary heart disease. Thus Wibell (6) demonstrated favorable effects on nitroglycerin consumption and pain incidence in a double-blind cross-over study on 16 patients with coronary artery disease, in which terodiline was matched against a long-acting nitroglycerin preparation, Graf (1) and Astrand (7) found some with improved exercise tolerance and reduction of ischemic ST-changes during or after exercise in a small group of patients studied with exercise electrocardiography, one hour before and 70 minutes after an oral dose of 25 mg terodiline.

The clinically recommended dose of terodiline is 25 mg t.i.d. per os. With this dosage, blood levels of terodiline equal to those one hour after an intravenous injection of 75-100 mg are expected within 48 hours of therapy.

The aim of the present investigation was to study the hemodynamic effects of intravenously injected terodiline by means of cardiac catheterization. The study was made on patients with angina pectoris produced by light physical exercise.

MATERIAL AND METHODS

Ten males between 40 and 64 years of age, all with typical clinical electrocardiographic and coronary angiographic signs of coronary heart disease, were studied. The studies were performed as part of pre- or post-operative evaluation of their coronary and myocardial status before or one year after surgery aimed at improving their myocardial blood supply. This evaluation included a careful medical history, exercise tolerance tests with ECG recordings and coronary angiography. None of the patients had signs of congestive heart failure or rheumatic heart disease and all had a sinus rhythm at the time of the study.

The studies were made in the morning when the patients were in the postabsorptive basal state. Right heart catheterization was performed with a double-lumen catheter and the brachial artery was catheterized using percutaneous technique. Cardiac outputs were measured by the indicator dilution technique using bromosulfalein injected via the right heart catheter into the pulmonary artery. Inductance manometers and a four-channel manograph (AB Elema, Stockholm, Sweden) were used for registration and recording of pressures. The patients were studied in the supine position. Exercise tests were performed on a bicycle ergometer (AB Elema).

Tensions of oxygen and carbon dioxide and pH in blood samples were measured with conventional electrodes (Instrumentation Laboratories Inc.) and oxygen contents were calculated using the Severinghaus nomogram (3).

Table 1 Hemodynamic data and differences at rest and during previous administration of terodiline with statistical analysis and probability (P) of obtained results

Case no	Dose	Experi- mental condition	Heart rate (beats/ min)		Stroke volume (ml)	Q (l/min)		a-vO ₂ diff (ml/100 ml)		F _{PA} (mm Hg)		F _{SA} Systolic (mm Hg)		P _{SA} Diastolic (mm Hg)		P _{DA} (mm Hg)		Pulm vase resistance		Syst vase resistance		LVSW (P)	
			A	B		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
			Rest	Diff		Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff	Rest	Diff
1	50 mg terodiline	300 kpm	60 ±0 +4	78 ±1 +4	97 ±2 -23	58 ±1 +4	45 ±1 +4	43 ±1 +4	48 ±1 +4	10 ±1 +4	13 ±1 +4	166 ±1 +4	121 ±1 +4	136 ±1 +4	0.5 ±0.1 +0.1	23 ±0.1 +0.1	11 ±0.1 +0.1	12 ±0.1 +0.1	156 ±1 +4	128 ±1 +4			
2	50 mg terodiline	150 kpm	67 ±2 -8	81 ±2 +4	68 ±1 +3	50 ±2 -8	53 ±2 -8	32 ±2 -8	34 ±2 -8	10 ±2 -8	12 ±2 -8	143 ±2 -8	87 ±2 -8	90 ±2 -8	1.2 ±0.1 +0.1	15 ±0.1 +0.1	20.4 ±0.1 +0.1	21.3 ±0.1 +0.1	101 ±1 +4	114 ±1 +4			
3	50 mg terodiline	250 kpm	68 ±1 +5	74 ±1 +5	94 ±9 +2	64 ±1 +5	77 ±1 +5	41 ±1 +5	45 ±1 +5	8 ±1 +5	12 ±1 +5	149 ±1 +5	81 ±1 +5	90 ±1 +5	0.6 ±0.1 +0.1	12 ±0.1 +0.1	15.9 ±0.1 +0.1	15.1 ±0.1 +0.1	120 ±1 +4	176 ±1 +4			
4	75 mg terodiline	450 kpm	66 ±1 +10	70 ±1 +10	96 ±19 +2	63 ±1 +10	54 ±1 +10	49 ±1 +10	44 ±1 +10	5 ±1 +10	12 ±1 +10	154 ±1 +10	82 ±1 +10	82 ±1 +10	1.1 ±0.1 +0.1	0.9 ±0.1 +0.1	18.4 ±0.1 +0.1	19.4 ±0.1 +0.1	145 ±1 +4	102 ±1 +4			
5	75 mg terodiline	300 kpm	80 ±0 +10	106 ±0 +10	108 ±10 +14	87 ±0 +10	94 ±0 +10	28 ±0 +10	38 ±0 +10	3 ±0 +10	9 ±0 +10	173 ±0 +10	82 ±0 +10	77 ±0 +10	0.5 ±0.1 +0.1	0.6 ±0.1 +0.1	12.3 ±0.1 +0.1	11.7 ±0.1 +0.1	153 ±1 +4	172 ±1 +4			
6	50 mg terodiline	600 kpm	66 ±1 +14	80 ±1 +14	68 ±2 -2	45 ±1 +14	53 ±1 +14	39 ±1 +14	36 ±1 +14	9 ±1 +14	12 ±1 +14	75 ±1 +14	30 ±1 +14	65 ±1 +14	0.9 ±0.1 +0.1	0.6 ±0.1 +0.1	10.7 ±0.1 +0.1	15.5 ±0.1 +0.1	40 ±1 +4	66 ±1 +4			
7	50 mg terodiline	300 kpm	58 ±1 +16	74 ±1 +16	59 ±15 +8	53 ±1 +16	59 ±1 +16	39 ±1 +16	38 ±1 +16	10 ±1 +16	17 ±1 +16	160 ±1 +16	73 ±1 +16	73 ±1 +16	1.7 ±0.1 +0.1	1.7 ±0.1 +0.1	18.3 ±0.1 +0.1	18.1 ±0.1 +0.1	109 ±1 +4	107 ±1 +4			
8	100 mg terodiline	100 kpm	96 ±1 +4	100 ±1 +4	96 ±9 +11	96 ±1 +4	75 ±1 +4	44 ±1 +4	52 ±1 +4	1 ±1 +4	10 ±1 +4	170 ±1 +4	117 ±1 +4	125 ±1 +4	1.4 ±0.1 +0.1	1.0 ±0.1 +0.1	21.5 ±0.1 +0.1	24.9 ±0.1 +0.1	19 ±1 +4	111 ±1 +4			
9	50 mg terodiline	400 kpm	88 ±1 +12	102 ±1 +12	87 ±11 +13	88 ±1 +12	83 ±1 +12	40 ±1 +12	43 ±1 +12	9 ±1 +12	12 ±1 +12	133 ±1 +12	72 ±1 +12	88 ±1 +12	0.3 ±0.1 +0.1	1.2 ±0.1 +0.1	14.9 ±0.1 +0.1	16.0 ±0.1 +0.1	16 ±1 +4	158 ±1 +4			

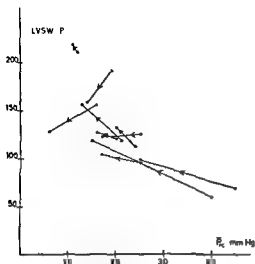


Fig 1 Changes in pulmonary capillary venous pressures (\bar{P}_{pc}) and left ventricular stroke work (LVSWS) during exercise induced by terodiline in 10 patients with coronary insufficiency

The simultaneous changes in capillary venous pressure and left ventricular stroke work during exercise induced by terodiline are illustrated in Fig 1. This comparison demonstrates that in eight out of ten patients the estimated left ventricular stroke work during exercise was unchanged or increased at a lower left ventricular filling pressure after administration of terodiline.

DISCUSSION

The results of this small hemodynamic study would indicate that acutely intravenously administered terodiline has effects upon the central hemodynamics during exercise in patients with coronary heart disease. The most striking effect is the lowering of the PCV pressure which indicates improvement of left ventricular failure. This effect is definitely different from that encountered after the administration of a placebo in a similar situation (2). The shift to the left and upwards in the relation between filling pressure of the left ventricle (pulmonary capillary venous pressure) and left ventricular stroke work in the majority of the cases indicates improved left ventricular function during exercise. This effect is similar to that induced by digitals administered in a similar fashion (2).

The myocardial function in patients with coronary heart disease may be altered by changes in

myocardial contractility, oxygen utilization, oxygen demand or oxygen supply. It is not yet fully clear which of these theoretical modes of action applies in the case of terodiline, although the results of animal experiments indicate that it should increase myocardial blood supply.

Our findings demonstrate that after administration of terodiline there were no untoward subjective effects and there was a definite subjective improvement in four cases. Objectively measured improvement was noted in eight out of ten patients, some of them very striking. On the basis of these results obtained with intravenous administration we believe that this drug should be tried as an adjunctive also in oral long term therapy of angina pectoris.

ACKNOWLEDGEMENTS

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LACTOSE MALABSORPTION IN GREENLAND ESKIMOS

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Abstract The incidence of lactose malabsorption in 37 Greenland Eskimos has been studied by means of sugar tolerance tests and in nine cases determination of disaccharidase activity in jejunal biopsies. Twenty three (77%) had lactose malabsorption. If seven Eskimos with a Danish ancestor are excluded the incidence of lactose malabsorption is 88% (22 of 25).

Lactose malabsorption seems to be a distinctive racial feature in Eskimos. Clinical signs of lactose malabsorption were rare but so was milk consumption in general. However, reactions to lactose ingestion during a lactose tolerance test were less pronounced than in lactase deficient white patients.

Man's ability to retain small intestinal lactase activity for the rest of life after the weaning period is extraordinary within the class of mammals. There are however exceptions since racial occurrence of lactase deficiency has recently been described in various areas of the world. It may reflect the fact that in man's history domestication of cattle and pastoralism with easy access to milk after weaning were introduced at different times in different parts of the world (13).

The incidence of lactose malabsorption in Eskimos has so far not been studied. If a connexion exists between the evolution of husbandry thousands of years ago and adult intestinal lactase activity one might expect lactase deficiency to be frequent in Eskimos. Not until the last few decades have dairy products been obtainable in the polar deserts.

We here report studies of lactose absorption in a limited number of Greenland Eskimos. Our results suggest that the incidence of lactose malabsorption among Eskimos is much higher than in Western Europe and the United States.

MATERIAL

Thirty two Greenland Eskimos were studied. They were all admitted from Greenland to this hospital because of diseases which, in the great majority, were unrelated to the gastrointestinal tract. None had malabsorption or any other sign of small intestinal disease. Their mean age was 33 years (observed range 13 to 75 years). Sixteen were females, 16 men. Seven patients knew of a European Danish ancestor; the remaining 25 considered themselves "pure" Eskimos.

METHODS

Sugar tolerance tests A lactose tolerance test (LTT) was performed in each case. Blood glucose was determined in capillary blood by a glucose oxidase method before and 15, 30, 60, 90 and 120 min after oral administration of 100 g lactose dissolved in water (8). Similarly a glucose galactose tolerance test with 50 g glucose + 50 g galactose was performed in 21 cases and a sucrose tolerance test with 100 g sucrose in 17 cases.

Small intestinal disaccharidase activity In nine cases jejunal biopsies were obtained at or up to 40 cm distal to the ligament of Treitz. Lactase, sucrase and maltase activity were determined by Dahlqvist's one step method (11). All biopsies were normal on stereomicroscopy and routine microscopy.

RESULTS

Tolerance tests A flat blood sugar curve (i.e. less than 25 mg increase of glucose per 100 ml blood (8)) in the LTT was found in 22 of 25 Eskimos without Danish ancestors but in only one of seven who knew of a Danish ancestor (Fig. 1). A normal rise of blood sugar followed ingestion of glucose galactose and of sucrose in all examined cases.

Six of 23 Greenlanders with flat blood sugar curves in the LTT displayed no symptoms during

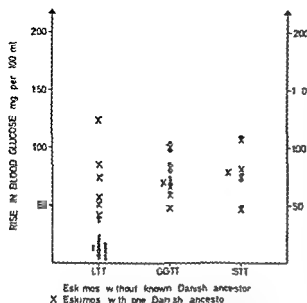


Fig 1 Maximum blood sugar rise in mg per 100 ml during lactose tolerance test (LTT), glucose galactose tolerance test (GGT) and sucrose tolerance test (STT) in 32 Greenland Eskimos.

the test. The remaining 17 had diarrhea and/or abdominal discomfort. The other tolerance tests produced no symptoms.

Jejunal mucosal lactase activity was abnormally in eight cases from 0 to 3 international units/g protein (normal figure > 9). The LTT was abnormal in seven. In the eighth who had diarrhea both after the LTT and when he drank milk the blood sugar rose by 30 mg per 100 ml.

A ninth patient had normal jejunal lactase activity. His LTT was normal too.

Mucosal sucrase and maltase activity was normal in every case.

Symptoms of milk intolerance. Nine patients complained of intermittent diarrhea. Eight of them had lactose malabsorption. All drank milk daily. Only two had observed that diarrhea followed milk intake and three had noticed abdominal cramps, bloating and flatulence after milk. Nevertheless they continued to drink milk.

Sixteen patients did not drink milk daily. Fourteen of them turned out to have lactose malabsorption.

Among 15 patients who drank milk daily lactose malabsorption was present in only nine.

Information on milk drinking habits is missing

in one case because of a temporary lack of an interpreter.

DISCUSSION

The incidence of specific lactose malabsorption (i.e. lactose malabsorption unassociated with other gastrointestinal diseases or with malabsorption of monosaccharides or other disaccharides) in white people in USA and Western Europe seems to be about 6% (1, 6, 7, 12) although in a few series lactose malabsorption occurred in 16 to 18% (9, 14). In contrast a much higher incidence has been found in other races: 70 and 73% in 20 respectively 41 American negroes (1, 4), 88% in 17 Greek cypriots (10), 89% in an African Bantu tribe (3), 100% of 11 Orientals (2) and 87% of 15 Chinese students (5).

In the present study lactose malabsorption was present in 23 of 32 Greenland Eskimos (72%). If only pure Eskimos are considered 22 of 25 (88%) had lactose malabsorption. The results indicate that lactose malabsorption is a distinctive feature in the Eskimo race. Since the Greenland Eskimos are supposed to be descendants of Canadian Eskimos who have been emigrants to Greenland over the last 4000 years, it is to be expected that a similar high incidence of lactose malabsorption prevails among Eskimos in Northern America.

The clinical significance of lactose malabsorption in Eskimos is difficult to assess for the moment. It will probably grow concurrently with an easier access to milk and milk products at distant villages along the 39 000 km coastline of Greenland. However it was remarkable to note that the clinical reaction to lactose during the LTT was absent or weak in many Eskimos with lactose malabsorption. Thus of 23 cases with lactose malabsorption only 11 had diarrhea and six had no complaints whatsoever. This is in sharp contrast to the severe reactions in the great majority of lactase-deficient European patients (7).

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EFFECT OF ANTI LEUKEMIC DRUGS ON CELL CYCLE OF HUMAN LEUKEMIC BLAST CELLS IN VIVO

PRELIMINARY REPORT

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It has become clear from clinical trials of chemotherapy in acute leukemia that the chance of inducing remission is less when a single agent is employed than when drugs are used in combination moreover results depend on how the agents used are applied (2) At the same time a general picture of the kinetics of leukemic blast cells in man is emerging (4) Adaptation of chemotherapy to the kinetics of leukemic cells may further improve therapeutic results If it can be defined where and for how long leukemic cells are inhibited in their progression through the cell cycle by various anti leukemic drugs this may provide a rational basis for selecting the optimal sequence and spacing of drugs Ultimately kinetic differences between leukemic and normal cells might be exploited to obtain more selective cytotoxicity Preliminary observations on the cell kinetic effects of prednisone and methotrexate are presented in this report

METHODS

After drug administration serial bone marrow samples are obtained from the patient and incubated with H thymidine (^3H TDR) for one hour (3) The percentage of ^3H TDR labeled cells (labeling index) = I_L is determined from autoradiographs Measurements of DNA content of individual cell nuclei and their ^3H TDR incorporation are done as follows (1) cells identified in Giemsa-stained preparations are plotted on photographic maps the stain is removed and, after Feulgen staining nuclear DNA is measured with the Zeiss UMSP I instrument The slides are processed for autoradiography the cells relocated and their ^3H TDR labeling determined

RESULTS

1 Effect of prednisone

A 21 year-old man with untreated acute lymphoblastic leukemia was given 200 mg of prednisone

in one oral dose and then 25 mg every 8 hours Immediately before therapy the ^3H TDR I_L of bone marrow lymphoblasts was 27.6 Sixteen hours later it had dropped to 5.8 and subsequently was reduced even further (Fig 1 (a)) The clinical effect was equally impressive and full remission was obtained by treatment with prednisone and vincristine When relapse took place the patient was put on the same prednisone regimen The ^3H TDR I_L of marrow lymphoblasts fell from 27.0 before prednisone to 6.6 24 hours after the first prednisone dose (Fig 1 (b)) I_L of myelocytes was not reduced The fall in I_L could not be ascribed to variable admixtures of circulating blast cells which have lower I_L than marrow blasts (3, 4) Fig 2 shows the DNA measurements of the blast cells Before prednisone 25% of the cells had a DNA content between 2n and 4n i.e. in the range of DNA replication Twenty four hours later 3% of the cells were in this range This is in good agreement with the ^3H TDR data and excludes the possibility that the drop in ^3H TDR I_L was due to a prednisone effect preventing DNA synthesizing cells from utilizing exogenous thymidine It is seen from Fig 2 that cells with a DNA content between 2n and 4n but not incorporating ^3H TDR are rare

Prednisone may lyse non proliferating leukemic lymphoblasts but the data show that prednisone at least in this patient acted preferentially on proliferating lymphoblasts This raises the question whether it is rational to administer primarily DNA synthesis blocking agents like methotrexate at the time of maximal prednisone effect on the cell cycle However this may depend on whether or not cellular methotrexate uptake is cell cycle

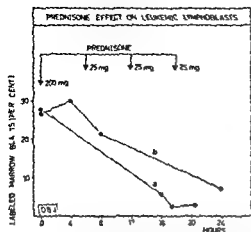


Fig 1 $H^3TDR I_L$ of bone marrow lymphoblasts in a patient with acute lymphoblastic leukemia after start of prednisone treatment a at diagnosis b at first relapse

phase dependent. This is being studied in this laboratory.

2 Methotrexate effect

Four patients with acute myeloblastic leukemia have been studied after a single intravenous dose of 15–20 mg of methotrexate. In all cases methotrexate was followed by rapid increases in the $H^3TDR I_L$ of marrow blasts which reached twice

the pre treatment values within 11 hours. Increases above the pre treatment level have been observed up to 24 hours. The results cannot be explained by variable contamination of circulating blasts nor by diurnal variations of I_L , known to be small or absent (5, 6). If methotrexate prevented progression through the DNA synthesis phase (S-phase) without affecting the normal entry rate into S, these data would suggest a DNA-synthesis time of approximately 8 hours. However, most in vivo studies indicate a considerably longer S-phase of leukemic myeloblasts (4). This raises the possibility that methotrexate may trigger off an acute extra recruitment of cells in pre-S-phase (G_1) into S-phase. One would then expect a piling up of labeled cells in early S during the first hours after methotrexate. Our microspectrophotometric data as yet incomplete show such a tendency; it is doubtful however whether this will quantitatively account for the rise in the $H^3TDR I_L$.

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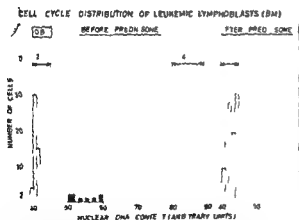


Fig 2 DNA content of leukemic lymphoblasts in bone marrow (same patient as in Fig 1) at time of first relapse. $2n$ and $4n$ are the diploid (granulocyte value) and tetraploid DNA values, respectively. 24 hours after initiation of prednisone cells with $> 4n$ DNA have virtually disappeared. The abscissa relating to the after prednisone cells has been cut since no cells with more than 48 arbitrary units of DNA were observed. Solid bars indicate cells labeled with H^3TDR , open bars represent unlabeled cells.

CATECHOLAMINES AND METABOLISM OF HUMAN ADIPOSE TISSUE

1 Comparison between *in vitro* Effects of Noradrenaline Adrenaline and Theophylline on Lipolysis in Omental Adipose Tissue

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Abstract Lipolysis in human omental tissue incubated in glucose-containing medium was determined by measuring glycerol release in the presence of different concentrations of noradrenaline and adrenaline. Almost identical responses were obtained with the two catecholamines. Significant increases in the glycerol release were obtained when they were added at concentrations as small as 0.03×10^{-6} M and maximal lipolysis was produced at $0.3-3 \times 10^{-6}$ M. Noradrenaline as well as 10^{-6} M of theophylline induced increases in the glycerol release were well correlated with the basal glycerol release.

Maximal glycerol release obtained with theophylline (10^{-6} M) was almost constantly six to eight times the basal glycerol release, whereas the lipolytic effect of noradrenaline was more variable and in no experiments exceeded significantly the theophylline effect. The results indicate that the formation of cyclic adenosine 3,5 monophosphate (3,5 AMP) or some process between the adenylyl cyclase receptor and the adenylyl cyclase system is rate limiting for the lipolytic response to noradrenaline. The data also suggest that variations in the lipolytic response to noradrenaline of adipose tissue removed from different subjects are principally caused by variations in the effect of noradrenaline on the formation of 3,5 AMP.

The present material of 32 patients does not allow critical analysis of the possible influence of sex, age and bodyweight of the donors on the tissue response to noradrenaline. However, the data indicate that adipose tissue obtained from old subjects has less lipolytic response than such tissue from young subjects.

The major role of the sympathetic nervous system in the control of lipid mobilization is well documented and has been reviewed by Havel (11), Wenke (26) and Himms-Hagen (14). A number of hormones have adipokinetic action in the rat and other low mammals (23). In human subjects by contrast only catecholamines (5, 8, 12, 19)

and glucagon (6) have been shown to have the property of increasing acutely the mobilization of FFA from the fat depots *in vivo*. It has been demonstrated by several authors (1, 2, 4, 7, 9, 16, 18, 20) that noradrenaline and adrenaline increase the release of FFA and glycerol from human adipose tissue *in vitro*. The activation of the catecholamine sensitive lipase demonstrable in human adipose tissue (1) is most likely mediated through the formation of adenosine 3,5 monophosphate (3,5 AMP) since theophylline in this tissue, as in rat adipose tissue, exerts a strong lipolytic effect (24). This is presumably due to inhibition of the phosphodiesterase activity and thereby prevention of the d-gradation of 3,5 AMP (3, 22, 25). Investigations of human adipose tissue have shown that the lipolytic response to noradrenaline as well as to adrenaline is rather variable (2, 4).

The purpose of the present investigation was to further investigate the potencies of noradrenaline and adrenaline in human adipose tissue *in vitro* and to study the reason for this variable response to noradrenaline. Future papers in this series of studies will deal with the effect of the catecholamines on the synthesis of triglycerides in human adipose tissue, the association between the formation and the breakdown of tissue triglycerides and the effect of adrenergic blocking agents on these processes.

MATERIAL AND METHODS

Omental adipose tissue was obtained from a total of 3 patients undergoing cholecystectomy. None of these

Table 1 Sex age and body weight distribution of subjects employed in this investigation

Sex	Ideal body weight ^a ()	Number of subjects		
		17-40 y	41-60 y	61-80 y
♂	81-100	2	4	6
	101-120	1	3	0
♀	81-100	4	2	3
	101-120	5	2	0

^a According to the Tables computed by Metropolitan Life Insurance Co (17)

tients showed evidence of diabetes mellitus or other metabolic disorders. Age, sex and ideal weight (17) of the donors are presented in Table 1. From the initial material of 34 consecutive patients two were later rejected in order to include only those patients who weighed within 80 to 110% of the ideal body weight. All patients were operated after overnight fasting. After induction with ultra short acting barbiturate general anesthesia was maintained with halothane and nitrous oxide in combination with suxamethonium chloride. The adipose tissue was removed at the start of the operation, placed into albumin containing buffer kept at 37°C and divided into pieces weighing about 50 mg each. After 60 min of precubation the tissue portions were transferred into Packard plastic vials containing 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) with the addition of 3 g/100 ml of bovine serum albumin (Armour Pharm Co, Eastbourne, England, Lot MG 2770) and glucose (100 mg/100 ml). The

ar albumin/FFA ratio was 1:3. About 200 mg of adipose tissue was incubated in each vessel. All incubations were run in duplicate. Aliquots of the medium were removed after two hours of incubation unless otherwise stated. Glycerol in the medium was determined by the enzymatic procedure of Wieland (27) as modified by Larsen (15). Handling of tissue and incubation procedure have been described in detail previously (28). In vitro additions of one or two of the following agents were made to the medium: adrenaline bitartrate and noradrenaline bitartrate (supplied as pure substances by Astra AB, Sodertälje, Sweden) and theophylline (obtained commercially).

RESULTS

Fig 1 shows that noradrenaline added at a concentration as small as 0.03×10^{-5} M (approximately 0.05 µg/ml) stimulated the glycerol release from omental adipose tissue incubated in the presence of glucose (1 mg/ml). This effect was statistically significant ($p < 0.001$). In all but one experiment a tenfold increase in this noradrenaline concentration resulted in maximal stimulation

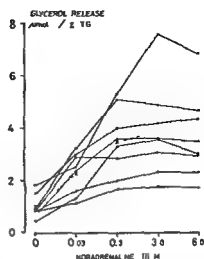


Fig 1 Omental adipose tissue (approx 200 mg) divided into sections of about 50 mg each was incubated for two hours in 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 3 (w/v) of bovine serum albumin and 1 mg/ml of glucose. Each point represents the mean of two incubations. The dotted line indicates the mean glycerol release from adipose tissue obtained from eight patients during surgical operation.

of the glycerol release. Lipolysis was neither accelerated further nor reduced by the addition of concentrations as high as 3×10^{-5} M and 6×10^{-5} M. In a single experiment adipose tissue showed marked lipolytic response to noradrenaline and the concentration of 3×10^{-5} M was required to give the maximal effect. The average increment in glycerol release produced by noradrenaline in these experiments was a fourfold increase (Fig 2).

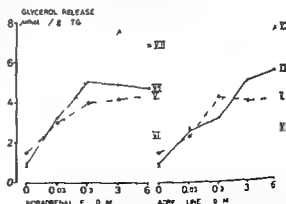


Fig 2 Comparison of the effects of noradrenaline and adrenaline on the glycerol release from omental adipose tissue in vitro. The incubation procedure is given in Fig. 1. Roman numerals refer to the serial numbers of the patients included in this investigation.

illustrates four experiments in which adipose tissue of each donor was incubated in the presence of either noradrenaline or adrenaline added at four different concentrations to the incubation medium. It was observed that the dose response curves for noradrenaline and adrenaline were rather similar and that the differences between the two agents were small and inconsistent.

Two types of experiments were undertaken in order to study which factor(s) might be rate limiting for the lipolytic response to noradrenaline. In the first series of experiments the rate of lipolysis was first studied as a function of time. We observed that the basal as well as the noradrenaline induced glycerol release was linear throughout two hours of incubation. From two typical experiments presented in Fig. 3 it is apparent that 0.3×10^{-5} M and 6×10^{-5} M of noradrenaline produced almost identical increases in the glycerol release determined after 10, 40, 70 and 130 min of incubation. In the second set of experiments the effect of adding a maximal dosage of noradrenaline (3×10^{-4} M) to the incubation medium was compared with (a) the effect of a maximal dosage of theophylline (10^{-3} M) and (b) the effect of both agents added together. The concentration of theophylline selected was one which had been found in previous studies to produce maximal stimulation of glycerol release from human omental tissue. These agents stimulated lipolysis by different mechanisms: noradrenaline increases the for-

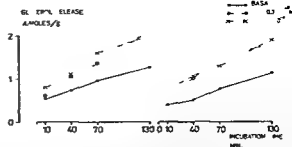


Fig. 3 Comparison of the effect of different concentrations of noradrenaline on the glycerol release from omental adipose tissue incubated 10, 40, 70 and 130 min in Krebs-Henseleit bicarbonate buffer containing bovine serum albumin (3×10^{-4} M) and glucose (1 mg/ml). ● basal ○ —○ NA 0.3×10^{-5} M × —×× NA 6×10^{-5} M ▲ —▲ NA 0.3×10^{-4} M

mation of 3,5 AMP (3, 22) whereas theophylline inhibits the degradation of this nucleotide (25). The results of these studies (Table II) indicate that the responses to theophylline were of similar magnitude since the increase in glycerol release varied from six to eight times the basal release in nine out of ten experiments. In five subjects (XI, XXVIII, XXXIX, XXXI, XXXII) it was observed that theophylline was a significantly more potent lipolytic agent than noradrenaline whereas in the other five experiments no differences between the agents were found. In these latter patients the addition of theophylline to noradrenaline-containing medium resulted in less than 50% increase

Table II Effects of noradrenaline, theophylline and both agents on the glycerol release from omental adipose tissue *in vitro*

Pat.	Glycerol release					
	Basal μmoles	Noradrenaline		Theophylline		Noradrenaline + theophylline μmoles
		μmoles ^a	per cent ^b	μmoles ^a	per cent ^b	
X	0.75	5.91	788	5.98	797	7.4 ^c
XI	0.53	1.51	285	3.82	721	5.2 ^c
XIV	0.84	4.56	543	4.00	476	4.6
XXVI	0.94	6.44	685	7.56	804	8.61
XXVII	1.27	6.69	527	7.76	611	9.66
XXVIII	1.05	3.56	339	7.60	724	9.39
XXIX	0.98	4.01	405	5.81	593	9.65
XXX	0.77	5.17	718	5.23	706	6.6
XXXI	0.99	2.36	238	6.43	655	11.0
XXXII	0.56	2.63	470	3.99	713	6.44

^a Calculated per g lipid weight \cdot h

^b Calculated as per centage basal lipolysis. Incubation procedure same as in Fig. 1

Noradrenaline conc. 3×10^{-4} M; theophylline conc. 10^{-3} M. Correlations: 1) basal versus noradrenaline-induced glycerol release $r=0.50$ $p>0.10$; 2) basal versus theophylline-induced glycerol release $r=0.84$ $p<0.005$; 3) basal versus noradrenaline + theophylline-induced glycerol release $r=0.6$ $p<0.05$

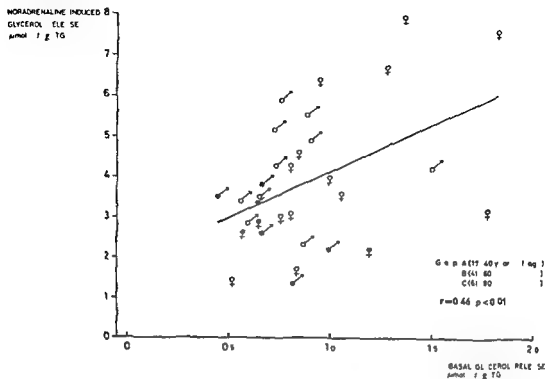


Fig 4 Relationship between the basal lipolysis and the glycerol release induced by noradrenaline (3×10^{-5} M) in

human omental adipose tissue. Correlation coefficient $r=0.46$ $p<0.01$. Regression equation $Y=2.29X+1.86$

(mean 27%) in the lipolysis produced by noradrenaline alone. By contrast theophylline had an additive effect on the lipolysis of adipose tissue from subjects showing a poor response to noradrenaline alone. In these patients the lipolysis in adipose tissue was increased more than 140% (mean 214%) by theophylline. Neither age nor sex of the donors seemed to be of importance for this difference in the response of adipose tissue to noradrenaline. The lipolytic responses to theophylline and noradrenaline plus theophylline were correlated with the basal lipolysis in this series of experiments. The correlation coefficients are presented in Table II.

The relationship between the effect of noradrenaline on lipolysis and basal lipolysis ($r=0.46$ $p<0.01$) is illustrated in Fig 4 in which the results of tissue obtained from all 32 patients are included. Since this small clinical material was rather heterogeneous with respect to age, sex and possibly also to amount of body fat it is not possible to ascertain whether any of these factors was of significance for the response to noradrenaline. However the results indicate that the lipolytic response to noradrenaline possibly

decreased with increasing age of the donor. Thus the glycerol release ($\mu\text{moles/g lipid weight/2 h}$) from omental adipose tissue from patients in group C (61–80 years of age) was 2.77 ± 0.76 (mean \pm standard deviation) and thus significantly lower than from tissue of patients in group A (17–40 years of age) (4.99 ± 2.03) $p<0.01$ and in group B (41–60 years of age) (3.74 ± 1.12) $p<0.05$. It is also evident from the figure that there were greater variations in the lipolytic response of adipose tissue from young than from older subjects.

DISCUSSION

Present data show that the lipolytic enzyme system of human omental tissue is remarkably sensitive to noradrenaline. Noradrenaline added in as low a concentration as 0.03×10^{-5} M consistently induced a marked increase in the glycerol release. In most experiments noradrenaline produced maximal response when added in a concentration of 0.3×10^{-5} M and with 3×10^{-5} M maximal effect was obtained in all experiments presented and undertaken so far. The lipolytic effect of nor

adrenaline and adrenaline in adipose tissue was of similar magnitude. The less sustained increase in the plasma FFA level after adrenaline administration compared with that after noradrenaline *in vivo* (10) is apparently due to the simultaneous effect of adrenaline on the glucose output from the liver and the subsequent inhibitory effect of blood glucose and/or insulin increase on lipolysis in adipose tissue.

Rate limiting for the *in vitro* lipolytic effect of noradrenaline and adrenaline is apparently the formation of 3,5 AMP in human omental tissue or some step between the adrenergic receptor site and the adenyl cyclase system. Neither the degradation of the hormones during incubation nor the metabolic changes evoked in tissues can be rate limiting since during the whole incubation period the lipolytic response of adipose tissue was almost identical at low (0.3×10^{-6} M) and high (6×10^{-6} M) concentrations of noradrenaline. In addition it was observed in all experiments that the glycerol release from adipose tissue was further enhanced either by theophylline alone or by theophylline plus noradrenaline. This would exclude the possibility that the capacity of the lipolytic enzyme system is rate limiting for the effect of noradrenaline.

It was of interest to observe that two different patterns of lipolytic response to noradrenaline and theophylline existed in the adipose tissue. The observation in five out of ten experiments that the effect of noradrenaline was significantly less than that of theophylline is best explained by the existence of some factor in these adipose tissue sections which decreases the ability of noradrenaline to accelerate the rate of formation of 3,5 AMP. In later experiments we have demonstrated that the lipolytic response to noradrenaline is stimulated in the presence of an alpha adrenergic blocking agent which suggests that the noradrenaline effect on lipolysis is antagonized by alpha adrenergic receptors (29). Our findings that theophylline had an additive effect on the noradrenaline induced lipolysis only in tissue showing poor response to noradrenaline cannot be explained until additional information about the relationship between 3,5 AMP and the lipase activity has been gained.

The present data as well as later experiments have shown that the maximal glycerol release produced by theophylline is well correlated with the

basal lipolysis and in nine out of ten experiments was six to eight times the basal release. Assuming that during lipolysis no net consumption of 3,5 AMP takes place these results would indicate that the percentage degradation of 3,5 AMP produced under basal conditions was rather constant for omental tissue removed from the present group of patients. In contrast to other authors (4) we were able to detect a positive relationship between the basal glycerol release and the noradrenaline induced lipolysis in human omental tissue. One possible explanation for the discrepancy between the results might be a difference in the composition of the clinical materials.

Our data indicate that there is some age dependency in the response of omental tissue to noradrenaline with the magnitude of response inversely correlated with increasing age. Other authors have attempted to correlate noradrenaline response in human adipose tissue with obesity (13, 18, 21). Such studies will require large numbers of subjects to distinguish the influence of obesity *per se* from the influence of unrelated factors such as age and sex.

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GLUCOSE TOLERANCE AND PLASMA INSULIN IN MAN DURING ACUTE AND CHRONIC ADMINISTRATION OF NICOTINIC ACID

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Abstract Since nicotinic acid is known to interfere with the glucose metabolism a detailed study was made to test whether insulin secretion is involved in this action. On chronic administration of nicotinic acid to hyperlipidemic subjects the fasting blood glucose and plasma immunoreactive insulin (IRI) concentrations rose significantly. The elimination rate constant (k_e) of intravenous glucose decreased from an average of 1.88 to 1.07. The plasma IRI response increased but only in relation to blood glucose level. Biguanide treatment did not improve the glucose utilization during nicotinic acid treatment.

A short term effect of the drug given as intravenous infusion to control subjects was a decrease of K value of iv glucose, a slight increase of basal plasma IRI level and an unchanged plasma IRI response to glucose load. In subjects maintained on total caloric restriction or on high fat low-carbohydrate diet nicotinic acid did not cause any further decrease of the primarily low K value. A similar lack of effect was present in patients with liver cirrhosis. Also in these instances the IRI response was not modified by nicotinic acid.

It is suggested that nicotinic acid impairs the hepatic glucose assimilation by increasing both glycogenolysis and gluconeogenesis through some action which is not related to antilipolysis. Insulin secretion is slightly stimulated by the drug.

Many reports (1, 2, 3, 25) have indicated that administration of nicotinic acid to normal or even diabetic human subjects causes an improvement of carbohydrate metabolism. In sharp contrast to this, however, the large doses of nicotinic acid used for the treatment of hypercholesterolemia have been rather convincingly demonstrated to result in an impaired glucose tolerance in non diabetic subjects (16) and to ameliorate diabetes mellitus (3, 4, 26). The reduction of glucose tolerance by nicotinic acid is a rather unexpected phenomenon in view of the fact that the drug inhibits lipolysis in adipose tissue and decreases the plasma FFA concentration (8) which should improve glucose

tolerance by increasing the glucose assimilation in muscle (37). Also in adipose tissue nicotinic acid at least in vitro increases the uptake of glucose (24). In fact nicotinic acid has been shown to accelerate the disappearance of tracer glucose in rats (38) and to abolish the impaired removal of glucose caused by noradrenaline infusion in man (28).

The mechanism by which nicotinic acid influences the glucose metabolism has not been solved. Lammers et al (22) have suggested that the impairment of the glucose tolerance might be due to an inhibition of the insulin secretion by the drug. Jenkins (21) thought that because ketone bodies stimulate insulin secretion (25) the reduced ketone body production during nicotinic acid leads to a diminished output of insulin and this accounts for the impaired glucose tolerance. To evaluate the role of insulin in the nicotinic acid induced changes of carbohydrate metabolism the effect of the drug was studied on the glucose tolerance and on the glucose stimulated plasma insulin response in hyperlipidemic patients treated with large doses of nicotinic acid and in normal fat fed and starved subjects as well as in patients with liver cirrhosis. The results show that the glucose tolerance is decreased by the drug but that this is not associated with any inhibition of insulin secretion.

MATERIAL AND METHODS

The chronic effect of nicotinic acid on glucose tolerance was determined in 14 hypercholesterolemic patients hospitalized for the investigation and treatment of their lipid disorder. Eleven of the subjects had a pure familial hypercholesterolemia (Type II) while the remaining three patients had in addition a slight hyperglycemia. The pa-

tients were kept on an ordinary hospital diet that provided about 40% of calories from carbohydrates. The control intravenous glucose tolerance test (IGTT 50 ml of 50% glucose) was carried out after the patients had been on the hospital diet for at least one week. Twelve of the subjects had normal k values while in the remaining two the fractional rate of glucose removal was less than 1.2. Nicotinic acid treatment was started with 1 g (Nicangin® Draco Sweden) on the first day 2 g on the second day and continued with 1 g three times daily. This treatment reduced serum cholesterol within two weeks by an average of 25% and triglycerides by 30%. After the maximal dose had been used for 10 to 14 days IGTT was repeated 14 hours after the last oral nicotinic acid dose and after 12 hours fasting. Plasma FFA concentration was measured at the beginning of IGTT and at 50 min after the glucose injection. Plasma immunoreactive insulin (IRI) was determined at 0, 2, 10, 20 and 50 min and the blood glucose at 10 minute intervals up to 50 min.

Oral glucose to errance tests (1 g of glucose/kg of body weight) were carried out in five of these patients before and during the nicotinic acid treatment.

To show whether biguanides are able to improve glucose to errance during nicotinic acid treatment the drug (Diaben retard® Astra Sweden) was given (50 mg twice a day) to seven additional hyperlipidemic non-diabetic subjects who had been on the nicotinic acid treatment for 10 days. IGTT was carried out prior to and 7 days after the start of the biguanide therapy.

The acute effect of nicotinic acid on glucose to errance
 was first studied in five metabolically normal inpatients who all showed normal k values in the IGTT. The test was carried out on consecutive days with and without prior nicotinic acid infusion (or vice versa) so that again every patient served as his own control. Nicotinic acid (Nicangin®) 200 mg for each subject was given as a slow intravenous injection over a period of 6 min. Blood samples for glucose, insulin and FFA analysis were drawn as indicated above and IGTT was started 30 min after the beginning of the nicotinic acid injection.

The same experiments were also carried out in patients given a high fat diet in starvation obese subjects and in patients with advanced cirrhosis of the liver. Five non-diabetic patients with 50 to 70% overweight were put on a total fast for 7-10 days. IGTT with and without nicotinic acid was carried out before and at the end of this period. The weight reduction ranged from 4 to 6 kg and all of the subjects showed a marked ketosis at the time of the second IGTT. Five other non-obese non-diabetic patients were put on an isocaloric diet that provided 80% of calories from fat (a mixture of soya bean oil and butter) 5% from carbohydrates and 15% from proteins. IGTT was performed before and after 7 to 10 days on this diet. An additional five patients with liver cirrhosis (two with Wilson's disease and three with excessive alcohol consumption) were studied. The diagnosis was verified from clinical condition and laboratory findings. The liver function appeared to be compensated in all but one subject whose serum bilirubin and ammonia were clearly elevated.

Analytical procedures The radio-iodination of insulin was carried out by the method of Hunter and Greenwood (14) and plasma IRI was determined according to Morjane and Lazarow (27). The orthotoluidine method by Hyvärinen and Nikkila (20) was used for the measurement of blood glucose. Triglycerides were determined by the method of Carlson (6). Cholesterol according to Pearson et al (33) and FFA as presented by Trout et al (40).

RESULTS

Chronic effect of nicotinic acid

The results are presented in Table I and they show that during the nicotinic acid treatment both fasting blood glucose and plasma IRI concentration increased significantly. The fasting FFA level was however not changed. The rate of glucose disposal as indicated by the k value of IGTT decreased in 12 out of 14 subjects and increased slightly in two. The k value which before the treatment exceeded 1.2 in 12 patients was during nicotinic acid treatment below that level in ten cases although none of them manifested clinical diabetes. The average k value was reduced from 1.88 to 1.07 by nicotinic acid.

That the decreased glucose utilization was not due to a deficient secretion of insulin is shown by the plasma IRI response which tended to be even higher during than before the nicotinic acid administration. However when the insulin response is expressed in relation to the corresponding blood glucose level i.e. as the ratio insulin/glucose (39) there appeared to be no difference between the on and off nicotinic acid periods. The average ratio at 2 min was 0.48 before and 0.45 during the therapy. The initial level of plasma FFA and its fall were of the same order in the two glucose tolerance tests indicating that the impaired glucose tolerance could not be accounted for by different availability of free fatty acids to tissues.

Since biguanides improve glucose utilization by peripheral tissues IGTT was carried out during chronic nicotinic acid treatment without and with additional biguanide therapy to show whether the drug is able to counteract the effect of nicotinic acid on glucose tolerance. As presented in Table II biguanide did not however significantly change the disposal rate of glucose although the plasma insulin response tended to be decreased by the drug. The ratio insulin/glucose was now smaller

Table I Effect of nicotinic acid treatment on fasting blood glucose on fractional removal rate (K) of intra-vascular glucose and on plasma insulin and FFA response to intravenous glucose (Mean \pm S.E.)

Treatment	Fasting blood glucose	K value	Plasma IRI response to intravenous glucose					Plasma FFA response	
			0 min	2 min	10 min	20 min	50 min	0 min	50 min
Control	84 \pm 2.3	1.88 \pm 0.24	16 \pm 2.2	64 \pm 9.2	51 \pm 5.2	39 \pm 4.2	32 \pm 5.2	576 \pm 75	787 \pm 35
Nicotinic acid ^a	93 \pm 2.0 ^b	1.07 \pm 0.11 ^b	25 \pm 3.3 ^b	85 \pm 9.9	64 \pm 6.1	57 \pm 4.4	41 \pm 5.3	565 \pm 72	268 \pm 21

^a Nicotinic acid 3 g/day at least for 10 days. The last dose was given 14 hours before IGTT (14 subjects)
^b $p < 0.05$

Table II Effect of biguanide (phenformin) treatment on the nicotinic acid induced changes of glucose and insulin metabolism (Mean \pm S.E.)

Treatment	Fasting blood glucose	K value	Plasma IRI response to intravenous glucose				
			0 min	2 min	10 min	20 min	50 min
Nicotinic acid	99 \pm 5	0.77 \pm 0.11	24 \pm 4	70 \pm 17	57 \pm 16	49 \pm 10	48 \pm 9
Nicotinic acid + biguanide	94 \pm 3	0.74 \pm 0.09	16 \pm 5	44 \pm 5	33 \pm 6	28 \pm 4	26 \pm 5

suggesting that glucose utilization per unit insulin was improved.

The oral glucose tolerance test was also impaired during chronic nicotinic acid treatment as revealed by Table III. Although the plasma insulin response to oral glucose was markedly increased during the therapy the ratio Δ insulin/ Δ glucose remained unaffected indicating that the insulin secretion was not influenced by the drug.

Acute effect of nicotinic acid in control subjects

Intravenous infusion of 200 mg of nicotinic acid to normal human subjects maintained on the ordinary hospital diet did not change the blood glucose level within 30 min but elevated the plasma IRI concentration (Table IV) and in agreement with earlier findings (8) decreased plasma FFA concentration (Table V) after an initial slight

but significant rise. IGTT carried out 30 min after the nicotinic acid injection revealed that despite reduced initial FFA concentration the glucose disappearance rate was significantly slower than with out nicotinic acid load (Table VI). The plasma IRI response expressed as the difference between basal and maximum levels was identical in both experiments (Table VII). The ratio Δ insulin/ Δ glucose calculated for 2 minute value (Table VIII) also showed that the actual insulin response was not influenced by nicotinic acid. Thus impaired glucose tolerance was not due to a poor stimulation of insulin secretion.

Table IV Effect of nicotinic acid infusion (200 mg) on fasting plasma IRI

The figures give the mean difference \pm S.E. (U/ml) from preinfusion value

Group (n)	Time (min)		
	10	20	30
Controls (5)	9.0 \pm 5.0	-7.0 \pm 3.4	8.0 \pm 1.9
Fasted (5)	-5.0 \pm 5	-5.4 \pm 9 ^a	7 \pm 1.9
Fat fed (5)	5.6 \pm 6.8	0.8 \pm 1.8	-4.5 \pm 1.5
Carbohydrates (5)	-4 \pm 1.4	-6.0 \pm 3 ^a	-5.4 \pm 7 ^a

^a Difference from control group significant at $p < 0.05$
 - number of subjects

Table III Effect of chronic nicotinic acid treatment on blood glucose (mg/100 ml) and plasma IRI (μ U/ml) during oral glucose tolerance test (Mean \pm S.E.)

	Difference from pretreatment values				
	0 min	30 min	60 min	90 min	120 min
Blood glucose	+15 \pm 1.1	38 \pm 4	14 \pm 7	9 \pm 4	8 \pm 3
Plasma IRI	+3 \pm 1	14 \pm 3	17 \pm 3	13 \pm 1	9 \pm 3

Table V Effect of nicotinic acid and subsequent IGTT on plasma FFA (Mean \pm SE)

Group	FFA μ Eq/l		
	0 min ^a	30 min ^b	80 min
Controls	335 \pm 25	251 \pm 24	228 \pm 45
Fasting	1181 \pm 168 ^c	816 \pm 98 ^c	1409 \pm 215 ^c
Fat fed	1019 \pm 142 ^c	569 \pm 102 ^c	779 \pm 256 ^c
Cirrhotic	523 \pm 115	261 \pm 48	340 \pm 100

^a Before nicotinic acid infusion^b Before glucose infusion^c Significant difference ($p < 0.05$) from corresponding control values*Acute effect of nicotinic acid in starved fat fed and cirrhotic subjects*

To learn whether the diabetogenic effect of nicotinic acid could be demonstrated also when the basal utilization of glucose was depressed the experiments were repeated in starved and fat fed patients. Particularly to show the role of the liver experiments were also performed in cirrhotic patients whose liver is known to form and utilize glucose less than normal.

The fasting blood glucose level was not changed in any of these groups during 30 min after the infusion of nicotinic acid. Basal IRI level was slightly decreased by nicotinic acid, the change at 30 min being in all three groups significantly different from that in controls (Table IV). Thus in contrast to normal subjects the basal utilization of glucose in relation to plasma insulin level appears to be improved by nicotinic acid in starved

fat fed and cirrhotic subjects. The FFA level, which in starved and fat fed subjects was originally higher than in the other groups, decreased significantly during nicotinic acid therapy in every instance but remained still higher than normal in fasted and fat fed patients (Table V).

Table VI shows that in contrast to normally fed control subjects the glucose disposal rate was uninfluenced by the drug in all these three groups. The average change of the k values differed significantly in starved and cirrhotic subjects from those in controls, suggesting that at least in these two conditions the factor was absent which normally is responsible for the acute impairment of glucose utilization by nicotinic acid load. Table VI demonstrates further that after nicotinic acid the k values were of the same order of magnitude in controls and in starved and fat fed patients despite 2-5 fold higher initial and final plasma FFA concentrations in the two latter groups (Table IV).

The response of plasma IRI in the IGTT was low after total fasting and high fat diet, the increment being at 2 min but not later significantly less than in normally fed controls (Table VII). The IRI response was not modified by nicotinic acid. In spite of still existing hyperinsulinemia the FFA level tended to increase at 50 min after the IGTT in subjects with superimposed nicotinic acid load (Table V) while low FFA levels were observed in patients without prior nicotinic infusion or with chronic nicotinic acid treatment (Table I). Although the ratio Δ insulin/ Δ glucose (which was especially low in starved subjects) was not significantly modified by nicotinic acid, the values at 2

Table VI Effect of nicotinic acid infusion on k values of IGTT

Case no	Controls		Starved		Fat fed		Cirrhotic	
	Before	After	Before	After	Before	After	Before	After
1	1.23	0.99	1.08	1.19	0.87	0.64	1.54	1.63
2	1.39	1.33	0.87	0.87	3.30	1.47	1.34	1.61
3	2.16	1.42	0.98	0.88	0.66	0.95	1.78	1.49
4	1.28	0.82	0.67	0.84	0.95	0.90	1.10	1.14
5	1.87	1.21	0.93	1.26	1.19	0.96	1.19	1.14
Mean \pm SE	1.59 \pm 0.17	1.15 \pm 0.11	0.90 \pm 0.08	1.02 \pm 0.08	1.39 \pm 0.48	0.98 \pm 0.13	1.39 \pm 0.13	1.41 \pm 0.09
Difference \pm SE	-0.43 \pm 0.13		+0.13 \pm 0.09 ^a		-0.41 \pm 0.36		+0.0 \pm 0.0 ^a	

^a Difference from control group significant at $p < 0.05$

Table VII Increment of plasma insulin (Δ IRI μ U/ml) during intravenous glucose tolerance test with and without superimposed nicotinic acid administration (Mean \pm SE)

Group	Nicotinic acid	2 min	10 min	20 min	50 min
Controls	+	69.0 \pm 13.7	29.8 \pm 6.6	18.6 \pm 7.7	12.2 \pm 2.8
	-	70.0 \pm 14.8	33.2 \pm 10.5	21.8 \pm 7.2	18.2 \pm 2.4
I	+	21.8 \pm 4.9 ^a	21.6 \pm 6.9	22.2 \pm 6.5	9.0 \pm 3.2
	-	17.6 \pm 3.7 ^a	11.4 \pm 4.0	9.6 \pm 2.0	10.2 \pm 5.6
I fed	+	30.2 \pm 9.0 ^a	21.6 \pm 6.4	13.6 \pm 4.9	11.0 \pm 3.6
	-	26.0 \pm 7.0 ^a	13.6 \pm 5.8	5.4 \pm 3.5	14.6 \pm 6.2
rhotoc	+	38.0 \pm 17.3	25.2 \pm 6.7	18.6 \pm 8.2	25.4 \pm 7.2
	-	51.0 \pm 23.3	49.8 \pm 32.2	79.4 \pm 57.5	111.4 \pm 11.5

^a Difference from controls significant at $p < 0.05$

n (Table VIII) were only about one fourth of in controls. The finding combined with the cal postnicotinate k values in control fasted fat fed subjects suggests that utilization of glucose per unit plasma insulin is markedly better after nicotinic acid in fasted and ed cases with high plasma FFA concentration in normal subjects with low FFA level.

DISCUSSION

The basic biochemical mechanism of the different actions of nicotinic acid is unknown. In adipose tissue it induces a very effective antilipolysis (5, 12) and increases the assimilation of glucose (19, 24) but it has not been shown whether these two effects are causally interrelated or have a common biochemical basis. Changes noted in other tissues and in blood may be secondary to these actions on fat cell but some of them may equally well be attributed to other primary pharmacological influences of the drug. Among the latter is the prompt decrease of liver and muscle glycogen (2, 38), reduction of blood and liver lipids (7) and variable response of blood glucose and glucose

tolerance. On account of the reduced plasma FFA level one should expect that the uptake of glucose in skeletal muscle is increased by nicotinic acid (37) but direct evidence of this has never been offered. Such an assumption is supported however by the finding that nicotinic acid enhances the RQ in both resting and exercising man (11, 18) and in dog (32). The finding that nicotinamide has a similar effect as nicotinate on blood glucose and liver glycogen (29) but has no effect either on lipolysis (5, 34) or on blood lipids actually suggests that nicotinic acid itself might have several primary metabolic actions.

The immediate effect of nicotinic acid on the fasting blood glucose concentration and on the elimination rate of a glucose load has been the subject of some controversy which however may be partly ascribed to different experimental conditions. Significant decrease of blood glucose level on administration of nicotinic acid have been recorded in fasted normal and diabetic rats (7, 9, 15, 38), in fasting diabetic dogs (32), in normally fed mice (2) and in diabetic human subjects (10). That insulin might function as one mediator in these effects of nicotinic acid is shown by the slightly but significantly increased plasma immunoreactive insulin level found here for human subjects and previously in rats (30). On the other hand no constant change of fasting blood glucose level of normal men was noted in the present study nor in an investigation of diabetics (17) nor by Paul et al (32) in normal fasting dogs. Also increases of blood glucose level on nicotinic acid infusion have been reported in normally fed and starving men (36).

Nicotinic acid in vivo accelerates the removal

Table VIII Effect of nicotinic acid infusion on the ratio Δ insulin/ Δ glucose at 2 min after intravenous glucose injection (Mean \pm SE)

Group	Before NA	After NA
Controls	446 \pm 086	452 \pm 087
Starved	167 \pm 061	102 \pm 043
Fat fed	245 \pm 084	118 \pm 034
Cirrhotics	231 \pm 094	190 \pm 136

^a Difference from control group significant at $p < 0.05$

and oxidation of glucose (32-38) which finding fits well to the effects observed in isolated adipose tissue or fat cells (19-24) and probably accounts for the fall of blood glucose. In non-diabetic subjects the increased utilization is compensated by an enhanced hepatic output of glucose which in fed state could come partly from stimulated breakdown of glycogen (24-38) partly from accelerated gluconeogenesis and in fasting or high fat feeding from the latter source only. The alterations of blood glucose level represent a net effect of all these three metabolic actions of the drug and this makes it understandable that rather variable changes of blood glucose have been reported. It is somewhat unclear whether nicotinic acid is able to enhance the peripheral uptake of glucose also in the absence of insulin. Root and Ashmore (38) observed an accelerated disappearance of labeled glucose after nicotinic acid in alloxan diabetic rats while Paul et al (32) could not demonstrate this in pancreatectomized dogs. Glucose entry rate was not stimulated in the latter instance either but was on the contrary decreased. The antilipolytic activity of nicotinic acid is not dependent on insulin (31).

In view of the influence of nicotinic acid on the basal turnover rate of blood glucose it seems difficult to understand why the disposal of exogenous glucose loads behaves just in an opposite way i.e. the elimination is retarded. The results of plasma insulin and FFA assays presented above indicate that neither a deficient insulin release nor a high FFA concentration can afford an explanation.

There is also no reason to assume that the assimilation of glucose by adipose tissue should be inhibited by some other mechanism causing insulin resistance. This is best shown by the observations in vitro mentioned above (19-24) and by the finding that in patients on chronic nicotinic acid treatment the plasma FFA response to intravenous glucose is normal in spite of a clearly retarded glucose removal. There thus remain the possibilities that glucose uptake by muscle or liver or both is inhibited. A defective glucose utilization in skeletal muscle after nicotinic acid could be ascribed to inhibition of muscle hexokinase by the increased production of glucose 6-phosphate from stimulated glycogenolysis but the rise of RQ in exercising man during nicotinic acid infusion (11) definitely tells against this possibility. The only

plausible explanation for the decrease of glucose tolerance is an impaired hepatic assimilation. This view is well supported by the absence of effect of nicotinic acid in cases with liver cirrhosis.

The factors other than blood glucose level and glucokinase activity determining the hepatic glucose uptake are not well defined. It seems however that stimulated gluconeogenesis and glycogen breakdown are accompanied by a decrease of glucose uptake by the liver even in the presence of hyperglycemia and active glucokinase. The primary point of action of nicotinic acid in both these processes has not been explored but the lack of its effect on glucose tolerance in conditions when glycogen stores have been exhausted and gluconeogenesis is primarily high—as in fasting and fat fed subjects of the present series and in diabetics studied by Carlson and Östman (10)—suggests that some rapid regulatory device switches the hepatic metabolism from glucose uptake to glucose production. On chronic feeding of nicotinic acid the same factor probably causes enzyme induction which leads to a constant hepatic overproduction of glucose, hyperglycemia and impaired glucose elimination which are no longer dependent on the actual presence of nicotinic acid in the circulation. That the enhanced glucose production did not appear to be related to actual availability of free fatty acid and that the same effect is obtained with non-lipolytic nicotinamide suggests that the primary gluconeogenic factor might be the changed nucleotide status in the liver. Nicotinic acid as well as nicotinamide results in a marked increase in hepatic NAD and NADP levels (13).

Note added to proof. Some of the results of the present study have been presented at the Meeting of the Scandinavian Society for the Study of Diabetes (Stockholm 1968 abstracted in *Diabetologia* 4:311 1968). Luyckx observed more recently (1969 personal communication) no effect of nicotinic acid on plasma IRI in anesthetized dogs but recorded a frank increase of the plasma glucose level.

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BLOOD VOLUME AND EXCHANGEABLE SODIUM DURING TREATMENT OF HYPERTENSION WITH GUANETHIDINE AND HYDROCHLOROTHIAZIDE

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Abstract Antipressor treatment with guanethidine for 8-14 days produces a significant average increase in blood volume and exchangeable sodium of 382 ml (8.5%) and 210 mEq (7.6%) respectively.

Subsequent supplementary treatment with hydrochlorothiazide resulted in a normalization of the above mentioned parameters both during short term and long term management.

It is proposed that these effects are of importance for the continued hypotensive effect of the thiazides.

It has been known for several years that a number of antipressor agents can cause fluid retention. This applies to ganglionic blocking agents (7, 22, 23), rauwolfia alkaloids (16) and α -methyldopa (10, 11, 15). Fluid retention is especially pronounced during treatment with guanethidine (4, 19, 20, 25) and a significant increase in blood volume (24, 26) and exchangeable sodium (26) results. The effects of thiazide preparations have been demonstrated in several studies which all show that exchangeable sodium, blood volume and extracellular space are reduced after treatment for less than 2 to 3 weeks (2, 3, 5, 9, 12, 27). The majority of these investigations show, however, that these parameters tend to become normal during long term treatment. According to Wilson and Freis (29), plasma volume and extracellular fluid reach pretreatment values in the course of 6-12 months of treatment. Other investigators find that plasma volume reaches the pretreatment level after 1 to 2 months of treatment (2, 9). Lauwers and Conway (18) could not demonstrate a reduction of extracellular fluid and exchangeable sodium after 26 to 60 days of treatment. In spite of this rehydration during long term treatment, Wilson and Freis (29) found that on discontinuance of the drug an addi-

tional increase in plasma volume, extracellular fluid and body weight occurred during the course of a week. This discontinuance took place on the average after 5.4 months of treatment.

One of us (11) on the other hand found a significant reduction in plasma volume and total blood volume after both 1 to 2 weeks and 3 months of treatment with hydrochlorothiazide in ten hypertensive patients without there being any sign of a decreasing effect on these parameters. Exchangeable sodium was also found to be significantly reduced after both short and long term treatment but here the effect seemed to be diminishing.

The objective of the present study has been to follow variations in total blood volume, extracellular space and exchangeable sodium during treatment with guanethidine alone and particularly during short term and long term combined therapy with guanethidine and hydrochlorothiazide, as such a study has not previously been undertaken.

MATERIAL AND METHODS

Total blood volume was calculated as the sum of the erythrocyte and the plasma volume using a double isotope technique with ^{51}Cr and ^{125}I albumin as previously described (13). Extracellular space and exchangeable sodium were determined with ^{22}Na which was given intravenously (approx. 100 μCi) after a standard had been taken. Activity in the serum and urine was measured after 3 and 24 h.

Extracellular space in liters was calculated from the 3 hour values, as these values are usable as an index of extracellular space (8). The following formula was used,

$$\frac{\text{administered activity} \times \text{activity in urine}}{\text{activity in plasma per ml} \times 1000}$$

Table 1 Exchangeable sodium, serum sodium, extracellular space, total blood volume, blood pressure and weight in 20 hypertensive patients during treatment with guanethidine alone and in combination with hydrochlorothiazide

Pat no	Sex	Age (y)		Before treatment	After 8-14 days treatment with guanethidine	After 8-14 days combined treatment	After 3-15 months combined treatment
1	o	39					(4 mo)
			TES	2861	3090	3108	3095
			TES/kg	37.7	39.2	38.9	37.5
			PS	141.0	142.0	141.0	140.5
			ECF	18.85	19.87	19.79	19.5
			BP	173	130	133	125
2	♂	40	W	75.8	78.9	79.9	82.5
			TES	3676	3571	3385	(5 mo) 3263
			TES/kg	47.7	45.4	44.5	38.8
			PS	145.5	141.5	140.0	147.0
			ECF	21.43	22.48	21.01	21.50
			BP	165	148	153	153
3	♀	70	W	76	77.5	76.1	84.0
			TES	2258	2617	2403	—
			TES/kg	31.3	35.6	33.4	—
			PS	140.0	146.0	147.25	—
			ECF	14.70	16.49	14.99	—
			BP	170	150	140	—
4	♂	65	W	71.7	73.5	71.9	—
			TES	3101	3168	3084	(3 mo) 2818
			TES/kg	52.3	51.3	52.3	49.4
			PS	145.0	145.0	144.5	147.0
			ECF	18.04	20.32	19.13	—
			TBV	4887	—	4067	4657
5		45	BP	160	140	125	153
			W	59.3	61.7	59.0	57.0
			TES	2185	2786	2132	—
			TES/kg	41.6	47.3	39.1	—
			PS	144.25	145.0	138.0	—
			TBV	3055	3371	3162	—
6	♀	58	BP	135	123	113	—
			W	57.5	54.1	54.5	—
			TES	3016	3065	2583	—
			TES/kg	39.3	39.9	34.9	—
			PS	147.25	145.0	139.0	—
			TBV	3933	4444	3711	—
7	o	54	BP	170	138	145	—
			W	76.7	76.8	74.1	—
			TES	310	3362	3169	(4 mo) —
			TES/kg	42.9	44.2	42.5	—
			PS	145.0	148.75	151.25	—
			TBV	5735	5197	5194	5168
8	♂	60	BP	153	138	120	138
			W	74.8	76.0	74.6	74.8
			TES	2959	3140	2844	(3 mo) 348
			TES/kg	47.0	48.7	46.1	44.5
			PS	143.0	145.0	143.75	145.5
			ECF	18.56	19.99	17.96	17.3
			TBV	4472	—	—	4349
			BP	193	143	135	175
			W	63.0	64.5	61.7	64.0

Table I Continued

Patient no	Sex	Age (y)		Before treatment	After 8-14 days treatment with guanethidine	After 8-14 days combined treatment	After 3-15 months combined treatment
9	♀	54					(5 mo)
			TES	2249	2420	2146	2230
			TES/kg	35.0	36.9	33.6	33.6
			PS	145.25	149.0	142.5	143.5
			ECF	13.53	15.01	13.73	14.23
			TbV	3439	3779	—	—
			BP	153	138	128	118
			W	64.2	65.5	64.2	66.3
10	♂	50					(9 mo)
			TES	2963	3093	2681	2766
			TES/kg	41.1	44.5	39.7	39.0
			PS	143.75	142.0	140.25	14.11
			ECF	18.23	19.54	16.98	17.45
			TbV	4651	5012	—	4413
			BP	185	170	150	155
			W	67.2	69.5	67.5	71.0
11	♀	47					(6 mo)
			TES	2887	3253	—	3078
			TES/kg	31.5	35.9	—	34.4
			PS	145.25	145.75	—	146.0
			ECF	17.92	20.44	—	18.74
			TbV	4662	4923	—	4921
			BP	130	135	—	110
			W	91.5	90.6	—	87.0
12	♂	58					(5 mo)
			TES	2884	3245	2864	2958
			TES/kg	42.3	46.0	41.3	39.7
			PS	138.5	140.0	141.0	140.0
			ECF	18.7	—	18.57	19.33
			TbV	4601	5273	4788	5146
			BP	170	148	143	155
			W	68.2	70.6	69.4	74.5
13	♂	63					(4 mo)
			TES	3502	4318	3315	3214
			TES/kg	46.8	55.0	44.7	40.4
			PS	145.0	141.25	140.25	140.0
			ECF	21.14	25.22	21.27	20.65
			TbV	5024	5844	5012	5128
			BP	163	145	140	143
			W	74.8	78.5	74.1	79.5
14	♂	59					(3 mo)
			TES	3083	3150	3011	930
			TES/kg	39.9	40.5	39.1	38.0
			PS	143.75	146.0	140.5	140.0
			ECF	18.81	19.78	18.71	—
			TbV	4515	4529	4291	4336
			BP	145	123	113	128
			W	77.2	77.7	77.0	77.1
15	♂	111					(11 mo)
			TbV	4986	5212	—	4777
			BP	138	118	—	130
16	♂	112					
			W	60.0	61.5	—	61.0
			TbV	4824	5005	4515	—
17	♂	113					
			BP	143	140	140	—
			W	66.4	66.8	66.8	—

Table 1 *Continued*

Pat no	Sex	Age (y)		Before treatment	After 8-14 days treatment with guanethidine	After 8-14 days combined treatment	After 3-35 months combined treatment
17	■	44	TbV	5641	6197	—	(7 m) 5341
			BP	148	143	—	138
			W	80.6	81.6	—	80.5
18	♂	61	TbV	5885	6365	—	(15 mo) 5947
			BP	153	143	—	148
			W	73.5	74.5	—	75.0
19		57	TbV	3786	4342	—	(13 mo) 3856
			BP	158	140	—	110
			W	54.5	55.5	—	56.4
20		45	TbV	3517	3795	—	(13 mo) 3675
			BP	130	115	—	105
			W	50.5	60.0	—	58.7

Abbreviations

TES—total exchangeable sodium in mEq

TES/kg—total exchangeable sodium per kg body weight

P_S—plasma sodium in mEq/liter

ECF—extracellular fluid in liters

TbV—total blood volume in ml

BP—blood pressure { $\frac{\text{systolic}}{\text{diastolic}}$ in supine position (mm Hg) }

W—body weight in kg

and in the same way exchangeable sodium was calculated from the 4-hour values.

$$\frac{\text{administered activity}}{\text{activity in plasma per ml}} \times \frac{\text{activity in urine}}{1000}$$

$$\frac{\text{activity in urine}}{\text{serum sodium in mEq/l}}$$

Serum sodium was determined in quadruplicate by flame photometry.

In cases in which blood volume, extracellular fluid and exchangeable sodium were determined the sodium determinations were done first and the blood volume measured immediately afterward. ²²Na activity was counted 1 to 3 days after the blood samples had been taken, while counting of the ⁵¹Cr activity was postponed for about a week to ensure that essentially all the ²²Na activity had decayed. All counts were done in a well-type scintillation detector and the counting of standards, blood, serum samples and background values was continued until the standard deviation for the total calculation was less than 2%.

Twenty patients with severe hypertension (1 men and eight women) were studied. Two had papilledema, three fresh exudates, and three had hemorrhages, while the others only had vascular eye-ground changes. None had heart failure before treatment. Renal function was estimated by means of creatinine clearance. 11 had greater

than 70 ml/min, three between 40 and 70 ml/min, and in five creatinine clearance was between 30 and 40 ml/min. The twentieth patient had a serum creatinine of 10 mg/100 ml. The patients were first treated with guanethidine alone in a dose of 1.5 to 100 mg per day for 8 to 14 days after which combination therapy with hydrochlorothiazide 25 to 75 mg daily was started. The addition of hydrochlorothiazide made it necessary in many cases to reduce the amount of guanethidine given in the first dose of the latter drug being thereby 10 to 20 mg a day. Studies were carried out before and after 8 to 14 days of treatment with guanethidine and were repeated after 8 to 14 days of combination therapy and again after 3 to 35 months of combined treatment. It was not possible to carry out all of the planned studies at all of the patients; in some cases only blood volume and exchangeable sodium measurements were done.

RESULTS

All of the relevant results are summarized in Table 1.

Blood volume

Average blood volume and blood pressure in 15 patients before and after short-term treatment with

Table II Mean blood volume (MBV) and the average mean blood pressure (MBP)^a in 15 hypertensive subjects before and after 8-14 days of treatment with guanethidine

	Before treatment	During treatment	Change (per cent)	SE	P
MBV (ml)	4517	4899	+382 (+8.5)	±68	<0.001
MBP (mm Hg) (supine)	152	135	-17 (-11)	±2	<0.01

^a Mean blood pressure = $\frac{\text{systolic} + \text{diastolic}}{2}$

guanethidine are shown in Table II. Treatment resulted in a highly significant average increase in blood volume of 382 ml accompanied by a significant fall in mean blood pressure of 17 mm Hg. Variations in blood volume in individual patients are shown in Fig 1. In only two cases was the blood volume essentially unchanged in the others it increased.

In seven patients the same parameters were compared after guanethidine treatment alone and after 8 to 14 days of combined guanethidine hydrochlorothiazide treatment. As shown in Table III there was a significant reduction in blood volume (454 ml) and a non significant fall in mean

Table III Mean blood volume and the average mean blood pressure in seven hypertensive subjects after 8-14 days treatment with guanethidine alone and after 8-14 days combined treatment with guanethidine and hydrochlorothiazide

	Guanethidine alone	Combined treatment	Change (per cent)	SE	P
MBV (ml)	4838	4384	-454 (-9.4)	±106	<0.01
MBP (mm Hg) (supine)	136	130	-6 (-4)	±3	>0.05

blood pressure (6 mm Hg). The reason for this is that it was necessary in several cases to reduce the dose of guanethidine in order to avoid too great a fall in blood pressure as earlier mentioned. Fig 1 illustrates variations in blood volume in individual patients. There was a marked reduction in six cases while blood volume remained unchanged in the rest.

Table IV (11 patients) shows that blood volume remains significantly reduced after long term treatment (3-15 months) with guanethidine and hydrochlorothiazide in relation to the values found during guanethidine treatment alone (316 ml) while there is no change in the level of blood pressure reduction. In four cases the fall in blood volume was less than 100 ml in the others it was more marked (Fig 1).

Our studies have also shown that blood volume remains unchanged after both long term and short term combined guanethidine hydrochlorothiazide therapy when the values obtained before treatment were compared to those obtained after.

Table IV Mean blood volume and the average mean blood pressure in 11 hypertensive subjects after 8-14 days treatment with guanethidine alone and after 3-15 months combined treatment with guanethidine and hydrochlorothiazide

	Guanethidine alone	Combined treatment	Change (per cent)	SE	P
MBV (ml)	5153	4837	-316 (-6.1)	±7	0.005
MBP (mm Hg) (supine)	136	134	-2	±3	0.5

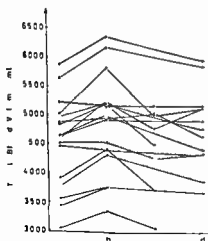


Fig 1 Total blood volume in 17 hypertensive subjects. (a) Untreated (b) During treatment with guanethidine (c) After 8-14 days of combined treatment with guanethidine and hydrochlorothiazide (d) After 3-15 months of combined treatment.

Table V Mean exchangeable sodium (MES), mean plasma sodium (MPS) and mean weight (MW) in 14 hypertensive subjects before and during short term treatment (8-14 days) with guanethidine

	Before treatment	During treatment	Change	SE	P
MES (mEq)	2913	3113	+210	± 59	<0.005
MPS (mEq/l)	143.75	144.45	+0.70	± 0.77	>0.30
MW (kg)	70.9	71.5	+1.60	± 0.32	<0.005

Extracellular fluid and exchangeable sodium

Exchangeable sodium increased significantly (mean increase 210 mEq or 7%, $p < 0.005$) in 14 patients during treatment with guanethidine alone and there was an average weight increase of 1.60 kg (Table V). As shown in Fig. 2 there was an increase in exchangeable sodium in all patients except one. The 3 hour ^{24}Na space was determined in ten patients and a highly significant increase of 1.83 l ($p < 0.001$) was found together with a mean weight increase of 1.70 kg. The increase in extracellular space varied from about 1 to almost 4 l (Fig. 3).

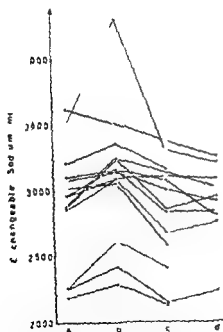


Fig. 2 Exchangeable sodium in 14 hypertensive subjects. (a) Unreated (b) During treatment with guanethidine (c) After 8-14 days of combined treatment with guanethidine and hydrochlorothiazide (d) After 3-9 months of combined treatment.

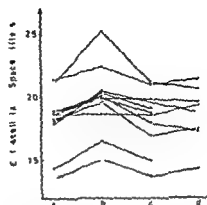


Fig. 3 Extracellular space in 11 hypertensive subjects. (a) Unreated (b) During treatment with guanethidine (c) After 8-14 days combined treatment with guanethidine and hydrochlorothiazide (d) After 3-9 months combined treatment.

In 13 cases conditions during guanethidine treatment alone were compared to those during short term guanethidine hydrochlorothiazide combination therapy (Table VI). The reduction in exchangeable sodium was significant (288 mEq, $p < 0.005$). Fig. 2 shows that exchangeable sodium was reduced in 12 patients and that in only one was there a small increase. In the nine cases in which the 3 hour ^{24}Na space was determined a mean reduction of 1.69 l ($p < 0.005$) was found with a mean weight decrease of 1.7 kg. In the individual patients there was a slight increase in extracellular space in one and a reduction of 1 to 4 l in the other eight (Fig. 3).

In ten cases exchangeable sodium after guanethidine treatment alone was compared to values after 3 to 9 months of combination therapy with hydrochlorothiazide. Table VII shows that there was a significant reduction of 331 mEq ($p < 0.01$).

Table VI Mean exchangeable sodium, mean plasma sodium and mean weight in 13 hypertensive subjects after 8-14 days treatment with guanethidine alone and after 8-14 days combined treatment with guanethidine and hydrochlorothiazide

	Guanethidine alone	Combined treatment	Change	SE	P
MES (mEq)	3113	2825	-288	76	<0.005
MPS (mEq/l)	144.39	143.87	-0.52	0.83	0.6
MW (kg)	71.1	69.6	-1.5	0.36	<0.005

Table VII Mean exchangeable sodium mean plasma sodium and mean weight in ten hypertensive subjects after 8-14 days treatment with guanethidine alone and after 3-9 months combined treatment with guanethidine and hydrochlorothiazide

	Guanethidine alone	Combined treatment	Change	SE	P
MES (mEq)	3240	2909	-331	± 92	<0.01
MPS (mEq/l)	143.80	142.68	-1.12	± 0.82	>0.20
MW (kg)	73.5	74.3	+0.8	± 1.07	>0.40

and a non-significant weight increase of 0.8 kg. Variations between individual patients are shown in Fig. 2. Three hour ^{24}Na space was determined in seven patients and a significant mean reduction of 1.87 l ($p < 0.02$) was found. Sodium space remained unchanged in one patient but decreased in the other six (Fig. 3).

These studies have also shown that the values for exchangeable sodium are the same before and after long term as well as short term combined therapy with guanethidine and hydrochlorothiazide. There is however a tendency to weight increase during long term combined therapy and since there is no change in extracellular space this is possibly a reflection of a true increase in total body mass.

Changes in serum sodium were on the average quite modest. Only when serum sodium values during guanethidine treatment alone are compared to those during short term combination treatment with hydrochlorothiazide is a significant reduction found ($2.52 \text{ mEq/l} \pm 0.83 \text{ mEq/l}$, $p < 0.02$ (Table VI)).

DISCUSSION

This study showed that there is an increase in blood volume, extracellular space and exchangeable sodium during guanethidine treatment. In contrast to Smith (26) we found that changes in blood volume and exchangeable sodium tended to be parallel. That this investigator did not find the same relationship may perhaps in part be due to the fact that he calculated blood volume on the basis of the plasma volume and the hematocrit value while in the present study the erythrocyte volume and the plasma volume were measured indepen-

dently with a double isotope technique. Determinations of the extracellular space perhaps indicated that weight increase is caused by an increase in extracellular fluid though this cannot be said with certainty without determinations of total body water.

The mechanism behind this salt and water retention is still not clear. Viasteris and Dustan (28) and Lauer et al. (17) believe that it is determined by an accentuation of the normal postural retention of sodium. This leads to salt and water retention during the day which must be compensated for by an increased excretion of these substances at night. If diurnal retention is greater than nocturnal excretion the net result is a retention of salt and water. According to Burkenhager et al. (1) this is particularly seen in patients with a marked endogenous diurnal excretory rhythm in whom nocturnal salt and water excretion may be insufficient. Smith (26) proposed the theory that guanethidine and other hypotensive drugs may stimulate the active tubular reabsorption of sodium. In this way salt and water retention would be independent of the effect these drugs have on glomerular filtration and renal blood flow.

In view of the effects of thiazide treatment in hypertension it is no surprise that combination therapy with guanethidine and hydrochlorothiazide produced a significant reduction in blood volume, extracellular space and exchangeable sodium. Previous studies have shown that there is a similar effect on blood volume when a thiazide is added to the therapeutic regimen of a patient being treated with a ganglionic blocking agent (23). Clinical experience with guanethidine and thiazides has shown corresponding effects in the form of weight loss and an increased excretion of sodium chloride and water (20). The agreement between weight loss and reduction of the extracellular space demonstrated in the present study seems to indicate that the most important homeostatic changes take place in that compartment.

We have found that hydrochlorothiazide induced reduction of blood volume and exchangeable sodium remains essentially unchanged even with long term (3-15 months) combination therapy. The explanation for this may be that guanethidine gradually loses its salt and water retaining effect. On the other hand Smith (26) has shown that the increase in blood volume and exchangeable sodium produced by guanethidine does not change greatly

during the first year of treatment. In addition clinical experience has shown that marked salt and water retention can appear at any time during treatment (20-25).

The mechanism by which thiazides produce their hypotensive and potentiating effects has not yet been explained. There is general agreement that the initial reduction of blood volume and possibly of extracellular space is the most important factor responsible for their effect with short term treatment (3-5-29). As mentioned above most investigators find that the effects on blood volume, extracellular fluid and exchangeable sodium disappear in the course of weeks to months (2-9-17-29). Thus other theories have been proposed to explain the long term hypotensive action of the thiazides e.g. that these drugs with long term treatment reduce peripheral resistance and thereby blood pressure by decreasing the salt and water content of the arteriolar walls (18). It is however likely that other factors also play a role. It has been shown for example experimentally as well as in man that chlorothiazide reduces the vascular response to noradrenalin (6-14).

The present investigation suggests that a reduction in blood volume, extracellular space and exchangeable sodium is also important in long term thiazide therapy in reducing blood pressure. This is in agreement with our previous findings (12). In addition this reduction could be demonstrated both in patients treated with thiazides alone and those treated with thiazide and guanethidine.

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THE POSTURAL PLASMA RENIN ACTIVITY RESPONSE IN ESSENTIAL HYPERTENSION AND PRIMARY HYPERALDOSTERONISM

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Abstract Supine and standing values of plasma renin activity (PRA) and colloid osmotic pressure (COP) were measured in 20 essential hypertensive patients including five malignant cases and in five patients with signs of primary hyperaldosteronism.

The groups were compared mutually and to 27 normal controls of corresponding age and sex.

Mean supine plasma renin activity (PRA) in essential benign hypertensives was significantly higher than normal. Mean supine PRA in malignant essential hypertensives was significantly higher than in benign essential hypertensives. Mean supine PRA in primary hyperaldosteronism was significantly lower than in benign essential hypertensives. In hypertensives with grade I-III a significant linear correlation between Δ plasma colloid osmotic pressure and Δ PRA appeared. The slope of the line was significantly less than the line for normal controls, signifying a lesser postural response of PRA in this group. In this respect there was no distinction between essential hypertensives and patients with primary hyperaldosteronism. The decreased postural PRA response is considered to be a sequel to the hypertensive state.

It has been established that plasma renin activity (PRA) in primary hyperaldosteronism is low or undetectable and the reaction to stimuli (e.g. changes in posture and/or salt depletion) is subnormal or completely abolished (3, 4, 5, 6).

It has been claimed that hypokalemia is no constant finding in primary hyperaldosteronism (6).

Provided that a suppressed response in PRA to changes in posture or salt depletion is adequately specific for primary hyperaldosteronism, PRA measurements during the above mentioned stimuli would be suitable as a screening test in the hypertensive population.

The answers given to this important question up to the present show the occurrence of a number

of false positives among patients with essential hypertension (7, 16, 23).

We have demonstrated a pronounced correlation between Δ PRA and hemoconcentration from supine to erect posture in normal individuals (20).

It was therefore thought of interest to study this correlation in essential hypertensives and in patients with primary hyperaldosteronism aiming at a more quantitative description of depression of the postural PRA reaction.

MATERIAL

Normal controls

Twenty-two individuals aged 19-67, five females and 17 males. The individuals were healthy hospital employees and patients hospitalized for minor conditions unrelated to the cardio-vascular or renal system.

Essential benign hypertensives

Sixteen individuals aged 22-63, two females and 14 males. These patients showed no signs of accelerated course of hypertension. "Symptomatic hypertension" was excluded by the following examinations: microscopy of urine sediment, intravenous pyelography, renal arteriography (if indicated), urinary excretion of vanillin mandelic acid, adrenaline and noradrenaline, urinary excretion of ketosteroids and ketogenic steroids, plasma electrolytes. There was no history of kidney disease or cardiac failure. Examination of the fundi showed grade I-III (Keith-Wagener). Anti-hypertensive treatment was discontinued at least 7 days prior to the examination.

Essential malignant hypertensives

Four individuals, aged 40-45, three females and one male. These patients had a severe progressive hypertension. The retinal changes were grade IV. As in the previous group any known cause of the hypertension was ruled out. All

Table 1 *Supine PRA (45 min recumbent) aldosterone secretion rate and plasma sodium and potassium after one week of normal salt intake (80 mEq Na⁺ 70 mEq K⁺ except case 25 investigated on liberal salt intake) and the same parameters after 4-6 days of salt loading (200 mEq Na⁺ 70 mEq K⁺ except case 47 who had 193 mEq K⁺) Three of the patients were investigated twice with an interval of 1-3 months*

Case no	Normal salt intake				Salt load			
	PRA ₀	Aldo secr rate (μg/24 h)	Plasma Na ⁺	Plasma K ⁺	PRA ₀	Aldo secr rate (μg/24 h)	Plasma Na ⁺	Plasma K ⁺
7								
1st invest	8	130	137	3.8	0	340	140	3.5
2nd invest	18	258	143	3.2				
17								
1st invest	10	453	146	2.8				
2nd invest	0	1464	147	3.2	0	308	144	3.0
25								
1st invest	4	208	143	3.7				
2nd invest	0	125	142	3.3	0	140	142	3.5
43								
1st invest	4	140	144	3.8	0	358	145	2.9
47								
1st invest	4	1937	145	1.8	0	558	145	3.1

the patients were hypokalemic (2.6-3.5 mEq/l). Drug treatment was discontinued 7 days prior to the examination.

Patients with signs of primary hyperaldosteronism

Five individuals aged 31-55, three females and two males. In all five patients suspicion was aroused either by spontaneous hypokalemia (cases 17, 43 and 47) or easily induced hypokalemia (gastroenteritis case 7 and diuretic therapy case 5). These patients are included in this group because 1) supine PRA was low or undetectable, 2) aldosterone secretion rate was increased at least in one of the determinations (>150 μg/24 h), 3) on salt load the aldosterone secretion rate was either not normalized or not suppressed if in normal range before salt loading (Table 1). These criteria are according to Luetjens (17). All patients had fundoscopic changes grade I-II, normal kidney function, there were no signs of a malignant course of hypertension, no signs of cardiac failure or symptomatic hypertension. Three patients have undergone surgery. In case 7 a bilateral nodular adrenal hyperplasia was revealed. The hyperplasia mostly included zona glomerulosa. The gland with the greatest changes was removed and the blood pressure is unchanged (6 months post-operatively). Case 17 revealed a left-sided adrenal adenoma. The adenoma consisted of zona glomerulosa tissue. The zona glomerulosa in the other adrenal gland was hypoplastic. Blood pressure is normalizing after three months of observation. In case 47 a left-sided solitary adrenal adenoma was found. Biopsy of the adrenal outside the tumor showed cortical atrophy. Blood pressure became normal a few days after surgery.

METHODS

An indwelling needle was placed in an antecubital vein. Coagulation in the needle was prevented by flushing it with small amounts of Na-citrate (3.8%). Immediately before blood samples were obtained 3 ml of blood was drawn and discarded to avoid citrate contamination from the needle. Subsequently blood samples were drawn into two disposable syringes of 10 ml each containing 1 ml 3.8% Na-citrate. The blood was transferred to a siliconized 50 ml Erlenmeyer flask immersed into ice water. Within one hour the blood was centrifuged at 3000 rpm for 15 min. The plasma was separated and kept at -20°C until renin activity was determined.

The PRA was determined by the method of Bouchet et al. with the modifications we have described (19) (coefficient of variance ±11.8%). The results are expressed as ng angiotensin/10 ml plasma/4 h incubation. In addition to the blood obtained for renin assay another 5 ml of blood was drawn into a dry syringe for determination of colloid osmotic pressure (COP), plasma sodium and plasma potassium. COP was recorded in an electronic osmometer for quick direct measurement of small samples as described by Hansen (13). The results are expressed in cm H₂O. Confidence limits 95% = ±0.5 mm Hg. Plasma volume in standing position as percent of value in supine position is determined as

$$\frac{\text{COP supine} \times 100}{\text{COP standing}}$$

Plasma sodium and potassium were determined by flame photometry. Aldosterone secretion rate was determined by

Table II Normal control on liberal salt intake The standing values are averages of 20 and 30 min samples

Liberal salt intake	PRA Supine (mean \pm SEM)	PRA Standing (mean \pm SEM)	COP Supine (mean \pm SEM)	COP Standing (mean \pm SEM)	Standing plasma vol in of supine (mean \pm SEM)
19 persons 17 males 5 females Range 18-67	15 \pm 2 Range 0-36 ^a	34 \pm 2 Range 15-60	34.8 \pm 0.6	41.2 \pm 0.7	84.62 \pm 0.96

^a Two persons showing 0

The double isotope method described by Kihman and Peter-son (15). The determinations were performed by Dr J Jacobsen Med Dept. B Rigshospitalet. The aldosterone secretion rate was determined on the same day as PRA. Postural studies were made in all patients and 20 normal controls on normal free salt intake all experiments being carried out between 8 a.m. and 11 a.m. When the patient had been in a supine position for 45 min the first blood sample was obtained as described previously (20). Knowing that a basal PRA level is obtained in normal subjects after this period of recumbency it was shown that the same applied to essential hypertensives by determining the values of PRA after 45 min and 7 h of recumbency in five hypertensive patients. In no case did the difference between the two determinations exceed the 95% confidence limit of the method. The patients were allowed to stand, position and permitted to take a few steps occasionally. Blood samples were obtained 5, 10, 20 and 30 min after the standing position had been assumed. In all hypertensives the blood pressure was measured several times at the end of the period in supine position. The mean of these determinations forms the mean blood pressure (diastolic pressure + 40% of the pulse amplitude) as shown in the Tables.

RESULTS

Normal individuals (Table II)

As described earlier by us (20) PRA reaches a plateau after 20 min of standing. The increase in PRA takes place between 10 and 20 min in the upright position. In all cases the 95% confidence limit is exceeded. COP increases fast after the erect position is assumed on an average 80% of the total increase takes place within the first 10 min of standing. The relation between Δ COP and Δ PRA is described by the following regression line $y = 2.8x + 1.0$ $r = 0.85$ $p < 0.001$ (Fig. 1).

Essential benign hypertensives (Table III)

The PRA in supine position is 25 ± 4 (SEM) which is significantly higher than in normal individuals ($t = 2.23$ $n = 36$ $0.05 > p > 0.025$). In

two patients (cases 30 and 32) PRA was above normal range but as in the other patients there were no signs of an accelerated or malignant course of hypertension. Mean PRA in standing position was 36 ± 4 which is not significantly different from normal individuals ($t = 0.44$ $n = 36$ $0.70 > p > 0.60$). No patients were clearly outside the normal range in the upright position. As shown in Table III there is no PRA increase in six patients (cases 2, 10, 18, 27, 30 and 32). In another six patients an increase in PRA does take place as in normal individuals (cases 9, 11, 15, 21, 22 and 36) while in two patients the PRA does not increase until after 30 min of standing (cases 3 and 46). The number of patients with a PRA increase exceeding the 95% confidence limit is significantly lower than in normal subjects ($0.02 < p < 0.05$). Table III demonstrates that all patients with eye background changes grade II show a significant rise in PRA while in only four out of 11 patients with grade I-III does PRA increase significantly. The difference between these two groups is not significant ($0.10 < p < 0.05$). The rise in COP takes place as in normal subjects. A plateau is reached after 10 min in erect position.

Plasma volume in the standing position in percent of supine plasma volume is 84.0 ± 1.3 which is not significantly different from normal ($t = 0.41$ $n = 36$ $0.60 < p < 0.70$).

Aldosterone secretion rate was determined in five patients. The values were normal except in case 2 where the secretion rate was increased but the corresponding PRA was in the upper normal range as well.

Essential malignant hypertensives (Table IV)

PRA in the supine position averages 148 ± 48 this is significantly higher than in patients with benign essential hypertension ($t = 2.6$ $n = 18$).

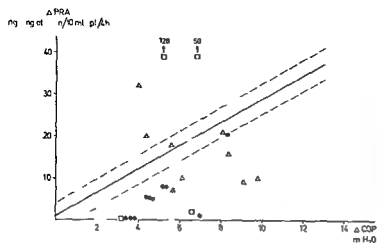


Fig 1 Correlation between increase in PRA and COP from resting values to 10 and 30 min of standing. The regression line for normal controls with standard error of estimate ($\pm 1 Syx$) is indicated. Essential hypertensives with grade 0 = Δ , Essential hypertensives with grade I-III = \bullet , Essential hypertensives with grade IV = \square .

Table III Essential benign hypertensives on liberal salt intake

Indices 0 = sample after 45 min supine 5 10 20 30 = minutes after assumption of standing position. Mean standing values are obtained as averages of 20 and 30 min values. These mean values are used in the computation of standing plasma volume in of supine and standing PRA in of supine (PRAI).

Case no	Sex	Age (y)	PRA ₀ COP	PRA ₅ COP	PRA ₁₀ COP	PRA ₂₀ COP	PRA ₃₀ COP	Mean stand PRA COP	Stand pl vol in of supine	PRAI ()	Mean BP FH	Aldo secre rate ($\mu\text{g}/24\text{ h}$)
2	♂	50	36	36	44	40	36	38	94.1	106	158	294
3	♂	22	39.9	43.7	43.6	42.5	42.2	42.4			11	
			18	21	—	28	39	34	83.9	189	126	—
9	♂	53	37.0	42.6	42.3	43.1	45.1	44.1			0	
			4	5	10	13	14	14	77.8	350	96	—
10	♂	63	33.5	38.6	40.9	42.6	43.3	43.0			0	
			27	24	—	28	36	32	83.8	118	154	174
11	♂	43	74.8	38.6	39.6	41.8	41.1	41.5			11	
			36	36	28	48	53	51	81.7	142	212	—
12	♂	44	31.0	41.5	43.1	43.9	44.0	44.0			11	
			13	—	20	21	10	16	88.5	123	160	103
15	♂	47	42.3	46.6	46.6	47.6	47.9	47.8			11	
			16	—	15	31	—	30	82.3	188	170	—
18	♂	57	72.2	38.1	39.7	37.7	40.4	39.1			11	
			33	—	—	22	28	25	91.0	76	144	—
20	♂	54	36.7	42.0	40.6	40.5	40.1	40.3			11	
			0	—	0	—	13	13	73.7	—	132	—
21	♂	37	35.0	46.6	47.0	46.9	48.0	47.5			11	
			33	—	36	65	51	58	88.0	176	120	—
22	♀	55	35.3	43.4	41.0	39.3	40.9	40.1			0	
			3	0	11	19	—	19	79.3	633	132	—
27	♂	52	32.0	35.5	37.9	40.4	—	40.4			0	
			31	—	34	40	41	41	77.5	132	123	—
30	♀	43	37.1	—	44.9	46.6	47.8	47.2			1	
			56	—	55	61	68	65	83.3	116	164	113
32	♂	48	38.0	—	43.3	45.6	45.6	45.6			?	
			50	—	70	60	58	59	84.5	118	149	—
36	♂	47	36.9	—	41.4	42.8	42.1	42.5			1	
			16	—	19	35	25	30	85.5	187	137	—
46	♂	22	35.5	—	38.8	42.0	41.0	41.5			11	
			29	—	31	36	49	43	88.4	148	1.4	—
			38.7	—	43.1	44.4	43.1	43.8			0	
Mean			25					36	83.95			
PRA \pm SEM			± 4					± 4	± 1.33			

Table IV Essential malignant hypertension

Indices - Table III

Case no	Sex	Age (y)	PRA ₀ COP ₀	PRA ₁ COP	PRA ₁₀ COP ₁₀	PRA ₂₀ COP ₂₀	PRA ₃₀ COP ₃₀	Mean stand PRA COP	Stand plasma vol in of supine	PRAI ()	Mean BP FH	Aldo secre rate (μ g \pm 4 h)
1	♀	20	96	—	—	—	109	109	—	113	180	—
3	♀	33	47	50	60	—	44	44	80.4	105	146	—
			30.5	34.5	36.3	38.6	37.1	37.9			IV	
1	♀	0	210	240	270	338	409	283	89.0	135	180	700
			34.6	39.0	40.1	40.0	37.8	38.9			IV	
5	♂	45	245	—	270	—	795	295	82.0	1.0	138	518
			30.7	—	33.9	—	37.5	37.5			IV	
Mean			148					183	83.8			
RA \pm SEM			± 48					± 63	± 6.4			

(0.02 > p > 0.01) In three patients PRA is above normal range in the supine as well as in upright position. The rise in PRA on assuming the standing position is not significant in any case. The increase in COP takes place normally and the plasma volume in the erect position in percent of the supine value corresponds to that of normal subjects ($r = 0.29$ $n = 24$ $0.80 > p > 0.70$).

Aldosterone secretion rate was determined in two patients both values being clearly increased.

Patients with signs of primary hyperaldosteronism (Table V)

PRA in the supine position averages 6 ± 2 which is significantly lower than in patients with essential hypertension ($t = 4.08$ $n = 21$ $p < 0.001$). Yet all values are within the range of essential benign hypertension. PRA in the upright position averages 13 ± 4 this is significantly lower than in essential benign hypertension ($t = 4.12$ $n = 21$ $p < 0.001$). All values are within the range of essential benign

Table V Patients with signs of primary hyperaldosteronism

Indices - Table III

Case no	Sex	Age (y)	Salt intake mEq Na mEq K ⁺	PRA ₀ COP	PRA ₁ COP	PRA ₁₀ COP ₁₀	PRA ₂₀ COP ₂₀	Mean stand PRA COP	Stand pl of of supine	PRAI ()	Mean BP FH	Plasma Na Plasma K
17	♂	53	80	10	6	35	23	29	74.2	290	18	146
17	♂	53	70	35.1	43.5	47.8	46.8	47.3			11	2.8
			80	0	0	4	—	4	8.6	—	141	147
7	♂	111	70	31.0	36.4	37.5	—	37.5			11	3.2
			Liberal	18	30	26	26	26	80.3	145	157	143
25	♂	111	Liberal	34.0	38.6	41.7	4.9	4.3			11	3.2
				4	11	15	11	13	83.6	3.2	136	143
25	♀	53	Liberal	30.4	33.4	37.1	35.6	36.4			1	3.7
				6	0	0	13	6	90.5	100	—	142
43	♀	43	111	33.0	36.6	35.0	37.4	36.4			1	3.3
			70	4	11	10	4	7	90.7	175	11	144
47	♀	43	80	36.1	37.9	39.4	39.7	39.6			1	3.8
			70	0	5	6	5	6	90.4	—	132	145
			70	4.7	44.8	47.1	47.4	47.3			11	1.8
Mean				6 \pm				13 \pm	84.6			
PRA \pm SEM				\pm				± 4	± 2.4			

Δ PRA
ng ang ot ns n/10 ml pl/6h

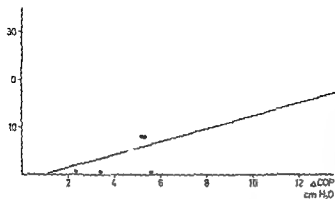


Fig 1 Correlation between increase in PRA and COP from resting values to 0 and 30 min of standing. Essential hypertensives with grade I-III = ● Primary hyperaldosteronism = ○

hypertension. In two patients (cases 25 and 43) the postural increase in PRA is not significant.

The plasma volume in the erect position in per cent of supine position is 84.6 ± 2.4 , which is not significantly different from normal subjects ($t = 0.08$, $n = 24$, $p > 0.90$).

Fig 1 depicts the relationship between Δ COP and Δ PRA from supine to plateau values for all hypertensives studied. It is seen that most of the values are below the regression line of normal subjects and outside the standard error of estimate ($\pm 3 \times s$). Correlation coefficient for hypertensives is $r = 0.09$, signifying no correlation. In Fig 2 values deriving from patients with mild hypertension (grade 0) and severe hypertension (grade IV) are omitted. The rest of the values demonstrate a pronounced linear correlation between Δ COP and Δ PRA. The regression line is $y = 1.4x - 1.2$ ($r = 0.68$). The correlation is significant ($t = 5.7$, $n = 32$, $p < 0.001$). This correlation line of hypertensive patients has a slope which differs significantly from the slope of the regression line of

normals ($t = 4.0$, $n = 64$, $p < 0.001$). The ordinate of the y axis intersection of the regression line is not significantly different from zero ($t = 0.63$, $n = 32$, $0.50 < p < 0.60$). Fig 3 demonstrates a scatter plot of corresponding values of supine PRA and standing PRA in per cent of supine PRA ($PRAI$) in normal subjects as well as in hypertensives. The values describe a clear curvilinear correlation and depict only one population.

DISCUSSION

In our group of essential benign hypertensives the mean values of PRA in the supine position are significantly higher than for normal subjects and the range is greater. This finding is in accordance with Meyer et al (18) while Helmer (14) and Pickens et al (21) have found lower PRA values in similar groups. Greco et al (11), Brown et al (1) and Weinberger et al (23) do not find values significantly different from normal individuals.

PRAI %

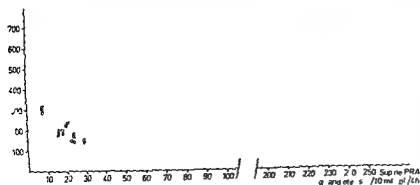


Fig 3 Correlation between PRAI (PRA in standing position in per cent of supine value) and the corresponding supine PRA value. Normal controls = ○ All hypertensives investigated = ●

these discrepancies may be due to different ratios of mild to severe hypertension in the materials. If the racial composition may also be of significance.

The malignant hypertensives have an increased supine PRA compared with patients with essential benign hypertension in correspondence with data from other investigators (2, 11, 12, 14, 18, 21 and 23).

The group of patients with signs of primary hyperaldosteronism show a mean PRA significantly increased compared with essential benign hypertensives. The values range from zero to normal, which corresponds to the findings of others (3, 4, 5 and 6) in clearcut cases of primary hyperaldosteronism. In all hypertensives the plasma volume depletion due to changes in posture from the recumbent to the standing position is within normal range, unlike the findings of Eisenberg and Wolf (9) who find an increased extracellular volume contraction in hypertensive patients.

Other investigators have shown a suppressed response of PRA to the assumption of upright posture in a fraction of essential hypertensive patients. While Warter et al. (22) and Weinberger et al. (23) as we do find a suppressed response in patients with high as well as low PRA, Gunnels et al. (12) and Creditor and Loeb (7) only find suppression in patients with low PRA. Kuchel et al. (16) by studying renin stimulation with diazoxide demonstrated that patients not stimulated by this drug have a decreased mean PRA compared with other essential hypertensives. Several of these nonresponding patients have undergone surgery and often no adrenal adenoma has been found.

In our investigation the increase of PRA in hypertensives on assuming the standing position is evaluated in different ways:

1. By indicating PRA in the erect posture in relationship to normal range. In this way no significant difference between normals and patients with benign essential hypertension and primary hyperaldosteronism can be found.

2. By indicating whether PRA response on assuming head-over-feet position exceeds the 95% confidence limit of the method. By using this criterion quite a number of hypertensives are distinguished from normals although not all.

3. By studying the relationship between supine PRA values and PRA1%. This relationship demonstrates that normal subjects and hypertensive

patients of all three types form one population. In this manner no pathological response is disclosed.

It is remarkable that PRA1% of high supine values is very close to 100% even in normals (Fig. 3). Accordingly the insignificant PRA response to the erect position in the three cases of malignant hypertension is not clearly pathological.

4. By studying the relationship between Δ COP and Δ PRA. Considering Δ PRA in relation to hemoconcentration (expressed as Δ COP) due to changes in posture (Fig. 1) it is seen that the great majority of the patients increase their PRA less than do normals with the same degree of hemoconcentration.

However, if the group of patients with fundoscopic changes of grades I–III is studied, a significant linear correlation is shown. The correlation line is clearly dissociated from the normal line by having a decreased slope.

By excluding patients with fundoscopic changes of grade 0 and grade IV the group formed is more homogeneous as to degree of severity. The fact that this group shows a uniform suppression in PRA response to postural changes does not elucidate the mechanism of the response. In the present state of our knowledge the suppression may as well be the result of the hypertensive state as it may be an indicator of a hemodynamic dysfunction leading to hypertension. Being uniform in degree of severity the patients of this group (grades I, II, III) may be divided into two subgroups.

One subgroup is made up of patients with signs of primary hyperaldosteronism. Three patients were submitted for surgery. Two were classical cases of Conn's syndrome. One patient revealed a nodular adrenal hyperplasia which has been described earlier in patients showing all features of primary aldosteronism (3, 8). The remaining two patients also meet the requirements of primary hyperaldosteronism but they have not undergone surgery for private reasons.

In the other subgroup it is reasonably assumed that the majority of the patients do not have primary hyperaldosteronism indicated by a normal to high PRA in the supine position. There is general agreement that cases of normokalemic primary hyperaldosteronism do not have PRA in this range (3, 4, 5 and 6). In five of the patients

Table I Results of tests on haemostatic function^a

Date	Bl T (sec) Ivy	Platelets (per mm) Feissly Ludin	Thrombelastogram					Coagulation factors activity						
			r (min) Hartert	k (min) Hartert	ma (mm) Hartert	P T (sec) Quick	T T (sec)	I (mg) Claus	II	V	VII	VIII One stage methods of normal plasma	IX	X
Case 2														
12 3 63	180	228 000	14	3	64	16 1	11 3	500	80	35	50			100
23 9 64	143	1 0 000	19	9	35	11 7	10	370						
21 7 67	172	157 000	23	10	42	12 0	9	390						
Case 3														
13 1 58	780	215 000					10 4		65	170	100			55
5 2 67	490	244 000	25	5	65	11 8	12 5	660				41	71	
5 10 62	>900	356 000	17	8	56	13 4	13 6	490				19		
27 4 64	696	268 000	17	7	56	14 2		325	100	100	100	30	100	100
26 9 66	>900		16	9	45	12 7		620	100	110	100	17	115	170
4 3 68												11		
Case 4														
27 10 61		110 000				14 7	<8	370						
29 2 68	<60	84 000	24	14	40	15 4	8 2	385		86				
Case 5														
22 6 66	180	52 000	30	18	33	17 9	12 2	130	75	30	60			50
8 7 66		169 000	20	18	30	15 3	14 2	180	80	40	60			70
26 7 66		110 000				15 8		580	90	110	90			100
13 2 67	252	100 000	19	11	60	14 9	12 4	860	60	95	75			
Normal values	117 ± 69	2 5 ± 0 5 10	18 0 ± 2 0	10 0 ± 2 2	47 0 ± 3 0	13 4 ± 1 4	15 ± 2 1	3 0 ± 40	Normal range 65-150					
(Mean ± S D)														

Bl T = bleeding time P T = prothrombin time T T = thrombin time

case 2 fibrin split products were absent in case 3 no anticoagulant against factor VIII was found

If tests performed by Dr J J Veltkamp)

with a planimeter. The base line was not obtained from a double sector cell but extrapolated from the horizontal part of the Schlieren curve in front of the fastest peak as it appears at the beginning of sedimentation. The relative percentage of a fraction is obtained in this way by putting the total surface at 100. Total protein and total macroglobulin are also given as absolute values which were calculated from the measured surfaces with the usual formula (27). A value of 0.000188 ml/mg for the refractive increment was calculated from an ultracentrifuge run of a purified IgM preparation: the protein content of which was determined by the Kjeldahl method. The macroglobulin content obtained by this procedure includes α_2 globulin as well as any normal heteropentameric IgM which may be present. The contributions made by these components were disregarded assuming that their variation during follow up studies was negligible. In one case of IgA paraproteinaemia (case 5) the level of the abnormal protein could be calculated by the same method since this component was mainly present in the form of fractions >7S. The contribution of 7S IgA paraprotein was disregarded in this case. Measurement by ultracentrifugation was preferred to Mancini's immunodiffusion method be-

cause this may give erroneous results when levels of different paraproteins have to be compared (14). The ultracentrifuge method gave a standard deviation of 0.03 g/100 ml for the single measurement of the macroglobulin peak.

Viscosity was measured with an Ostwald viscosimeter using undiluted serum at a temperature of 37°C. Corrections for specific weights were not applied since they were within the experimental error.

Methods used in blood coagulation studies are mentioned in Table I. For further references see Hensen and Loeliger (11) and Loeliger et al (19).

Immunofluorescence studies on bone marrow in case 5 were performed by the direct method using monospecific fluorescent conjugates and concentrated suspensions of washed cells instead of smears (9).

CASE REPORTS

Case 1

S, a 56-year-old man was referred to the hospital in September 1963 because of haemolytic anaemia which did

not sufficiently respond to prednisone treatment. Physical examination revealed pallor, petechiae on the lower legs, peripheral oedema and a slightly enlarged spleen. The eye grounds did not show any haemorrhages. Laboratory investigations: haemoglobin 7.1 g/100 ml, reticulocytes 0.4, leucocytes 6,000 per mm³ with a normal differential count, platelets 740,000 per mm³. The blood smear showed rouleaux formation of the erythrocytes. The ESR was 133 mm after one hour. Total serum bilirubin was 13 mg/100 ml with increased indirect reacting bilirubin. The Bence Jones reaction in the urine was negative. Results of all other routine laboratory tests were normal. The cytologic picture of the bone marrow smear revealed an increase in lymphocytic elements and erythroid hyperplasia. The direct Coombs test was positive; tests for cold agglutinins were negative. A Cr⁵¹ red cell survival gave a T_{1/2} of 8 days and scanning demonstrated an increase in splenic sequestration of erythrocytes. The total protein was 7.4 g/100 ml. Agar and immunoelectrophoresis of serum revealed an IgM paraprotein. In the ultracentrifuge a 19S macroglobulin content of 23% of the total protein was measured. A diagnosis of Waldenström's macroglobulinaemia with haemolytic anaemia was made and treatment with chlorambucil in a dose of 4 mg per day was instituted, while prednisone was continued in a dose of 40 mg/day. Three months later there was a subjective improvement. The direct Coombs test was still found positive. The course of other relevant laboratory investigations and of the therapy during the case history is represented in Fig. 1. After a nine months course of treatment with chlorambucil the haemoglobin had hardly changed, but a decrease in macroglobulin content of the serum was found. In November 1964 the prednisone was discontinued. One month later laboratory investigations disclosed a decrease in haemoglobin from 10 to 7.9 g/100 ml. The cytologic picture of the bone marrow smear showed marked erythroid hyperplasia. After reinstitution of prednisone the haemoglobin rose gradually to 10.8 g/100 ml. In December 1964 the chlorambucil was discontinued for several months because of a low white cell count. In November 1965 the patient complained of a tumour in the left inguinal fossa. Physical examination revealed unilateral inguinal lymphadenopathy. Cytologic examination of an inguinal lymph node revealed reticulum cell sarcoma. There was an increase in lymphocytic elements, mast cells and plasma cells in the bone marrow. The titre of the direct Coombs test had decreased. Ultracentrifugation of the serum showed that the macroglobulin level had risen. A chest X-ray and an abdominal lymphangiogram were normal. In January 1966 splenectomy was performed in an attempt to treat the haemolysis. The patient died two weeks later of pulmonary embolism.

Case 2

A 70-year-old man was admitted to the hospital in January 1963 because of fatigue and haemolytic anaemia. He was treated with prednisone in a dose of 0 mg per day. On this therapy his general condition deteriorated and he was referred to the University Hospital in Leiden in March 1963 for further treatment. On admittance pallor and jaundice were noted; the liver was felt 3 cm below the right costal margin and the spleen 4 cm below the

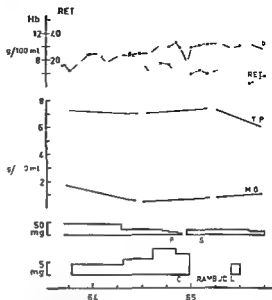


Fig. 1 Clinical course of case 1 (S). Hb = haemoglobin, RET = reticulocytes, TP = total protein, MG = macroglobulin. Time scale on abscissa.

left costal margin. The temperature was 39.6°C. Fundoscopic examination revealed no abnormalities. Laboratory investigations: haemoglobin 3.2 g/100 ml, erythrocytes 0.8 million per mm³, reticulocytes 4.9%, leucocytes 8,000 per mm³ with a normal differential count, thrombocytes 275,000 per mm³. The blood smear showed rouleaux formation of the red cells, marked anisocytosis, poikilocytosis, polychromasia and many normoblasts. The ESR was 176 mm after one hour. The total serum bilirubin was 5 mg/100 ml with increased indirect reacting bilirubin, and the LDH was 800 units. The urine contained excess of urobilinogen; the reaction to Bence Jones protein was negative. The cytologic picture of the bone marrow smear showed a marked erythroid hyperplasia and an increase in mast cells. The direct Coombs test was positive; the cold agglutinin test was negative. The total serum protein was 8.5 g/100 ml. Agar and immunoelectrophoresis of the serum revealed an IgM paraprotein which proved to be a cryoglobulin. In the ultracentrifuge a macroglobulin content of 34% of the total serum protein was measured. Investigation of haemostatic functions revealed some abnormalities (see Table I). X-ray examination of the skeleton showed no abnormalities. A diagnosis of Waldenström's macroglobulinaemia with haemolytic anaemia was made. Four units of packed red cells were transfused and the dosage of prednisone was increased to 60 mg per day. Thereafter his condition improved rapidly. The course of the disease was complicated by an infection with *Salmonella paratyphi D* which was successfully treated with ampicillin. By the end of March 1963 the haemoglobin and reticulocyte count values had considerably improved. The total serum bilirubin was 1.06 mg/100 ml. Treatment with chlorambucil in a dose of 6 mg per day was started and the prednisone dosage was decreased to 30 mg per

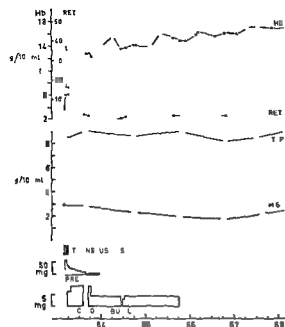


Fig 2 Clinical course of case 2 (B)

day Examination of a bone marrow smear showed focal erythroid hyperplasia a large number of mast cells an increase in lymphocytic cells sometimes seen in clumps and an increase in plasma cells For the course of laboratory investigations and of therapy see Fig 2 Five months later the patient was feeling well The titre of the direct Coombs test had decreased and was found to be negative

October 1965 The treatment with chlorambucil was used until October 1965 but the prednisone dosage gradually decreased and ultimately stopped by December 1963 During 1964 and 1965 the patient was free from symptoms In October 1966 the patient was found to be in a good general condition and a physical examination revealed no abnormalities In January 1967 the cytology of the bone marrow showed a decrease of lymphocytic cells compared with four years previously At the time of writing this article 28 months after the cessation of the chlorambucil treatment the patient is still feeling well

Case 3

G a 74 year-old man was first seen in 1958 because of a two years history of nosebleeds and chronic bronchitis Physical examination revealed no abnormalities The haemoglobin was 131 g/100 ml erythrocyte count 4.1 mill per mm reticulocytes 2.6 thrombocyte count 215 000 per mm ESR 34 mm after one hour total serum protein 8.6 g/100 ml Investigation of haemostatic functions showed a prolonged bleeding time see Table I Other routine laboratory tests were normal Roentgenological examination of the chest showed no abnormalities In April 1961 he visited the outpatient department complaining of frequent nosebleeds The haemoglobin value was 9.6 g/100 ml The patient was found to have coronary sclerosis and congestive heart failure Treatment consisted

of digitalis diuretics and oral iron In February 1966 he had many severe nosebleeds necessitating blood transfusions Laboratory investigations revealed a low activity of factor VIII (see Table I) A tentative diagnosis of vascular haemophilia (Willebrand's disease) was made although a family study gave negative results In March 1962 he was admitted to the hospital because of melena, probably caused by a sliding hernia of the oesophagus.

A haemoglobin value of 6.6 g/100 ml was found. In 1963 he had no complaints but in 1964 he again suffered from intermittent nosebleeds and fatigue On physical examination no abnormalities were found Laboratory investigations revealed haemoglobin 13 g/100 ml leucocyte count 5100 per mm³ serum calcium 9.9 mg/100 ml and total serum protein 6.9 g/100 ml The direct Coombs test was negative and the reaction for Bence Jones protein in the urine was also negative An IgM paraprotein was found in the serum by agar and immuno-electrophoresis. On ultracentrifugation it appeared that the macroglobulin fraction amounted to 10% of the total serum protein. Examination of a bone marrow smear revealed the preponderance of plasma cells many of which contained large Russell bodies A roentgenological examination of the skeleton showed no abnormalities A diagnosis of Waldenström's macroglobulinaemia was suspected. For the course of the relevant laboratory investigation and of the therapy see Fig 3 In December 1964 ultracentrifugation showed that the macroglobulin level was almost normal A treatment with chlorambucil was started in May 1965 During 1966 the patient had only a few nosebleeds. At the time of publication the patient is still on chlorambucil treatment He has few and small nosebleeds The haemostatic functions have not improved

Case 4

R a 69 year-old man was admitted to the hospital in August 1961 with a six months history of nosebleeds, bleeding gums anaemia and some blurring of vision

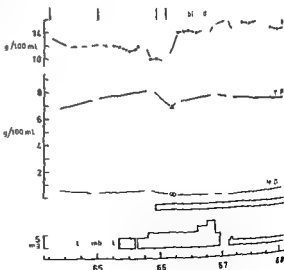


Fig 3 Clinical course of case 3 (G)

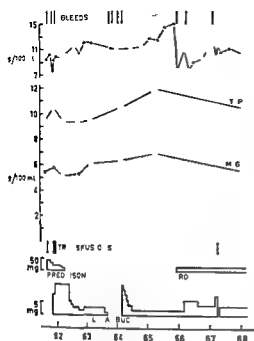


Fig 4 Clinical course of case 4 (R.)

(clinical data made available by Dr J F L Fonten) A month prior to his admission he had been examined by an ophthalmologist who found a distension of the retinal veins with multiple haemorrhages. The visual acuity was 10/10 in the left and 5/10 in the right eye. On physical examination the liver was felt 3 cm below the right costal margin. There was no splenomegaly. Laboratory data: haemoglobin 9.5 g/100 ml, reticulocytes 17%, thrombocytes 130 000 per mm³ and ESR 135 mm after one hour. The results of other routine laboratory tests were essentially within the normal range. Examination of a bone marrow smear revealed focal erythroid hyperplasia and an increase in lymphocytic cells. The total protein was 9.5. An IgM paraprotein was detected in the serum by agar and immunoelectrophoresis. A macroglobulin content of 57% of the total serum protein was measured by ultracentrifugation of the serum. A diagnosis of Waldenström's macroglobulinaemia was made. Two units of blood were transfused and a treatment with prednisone in a dose of 40 mg per day was instituted. The patient was readmitted in October 1961 because of severely bleeding gums and dizziness, possibly of vestibular origin. Now the spleen was felt 2 cm below the left costal margin. The haemoglobin was 7.2 g/100 ml. The results of the tests on haemostatic functions are presented in Table I. Six units of blood were transfused and treatment with chlorambucil in a dose of 8–12 mg was instituted. For the course of relevant laboratory tests and of the therapy see Fig 4. In March 1962 the patient had fewer episodes of bleeding from the nose and the gums and there was no splenomegaly. The prednisone was discontinued. During 1963 the patient was feeling well. The chlorambucil treatment was discontinued in September 1963. From October 1963 until March

1964 the patient suffered from intermittent bleeding from the nose and the gums. A treatment with chlorambucil in a dose of 1 mg per day was reinstituted. In May 1964 the patient had no complaints of bleeding. He remained free from symptoms until December 1965 when he again experienced severe bleeding from the gums and epistaxes, accompanied by a strong decrease in haemoglobin. A treatment with iron was instituted. Because of persisting bleeds the dose of chlorambucil was increased in March 1966. From May 1966 until March 1967 the patient only had intermittent very small nosebleeds. On March 8 1967 an operation performed because of a basaloma was complicated by haemorrhages. In September 1967 the patient complained of a total loss of vision of the right eye. He was seen by his ophthalmologist, who found an ablatio retinae. Fundoscopic examination also revealed hardly any improvement of the left eye: distended retinal veins and scattered haemorrhages were still present. On a return visit in January 1968 the patient had no further complaints. Physical examination revealed no abnormalities. Laboratory data in February 1968: The ESR was 139 mm after one hour, serum calcium 8.9 mg/100 ml, serum uric acid 7.4 mg/100 ml. The Bence Jones reaction was negative. The direct Coombs test was negative, cold agglutination was also negative. Investigation of haemostatic functions: see Table I. The patient is maintained on 4 mg chlorambucil daily.

Case 5

L., a 75-year-old woman, was admitted to the hospital in June 1966 because of severe epistaxes and diminished consciousness. Pallor was noted but further physical examination revealed no abnormalities. Laboratory investigations: haemoglobin 5.5 g/100 ml, erythrocyte count 1.6 mill per mm³, reticulocytes 6%, leucocyte count 7100 per mm³, platelets 32 000 per mm³, ESR 16 mm after one hour, serum calcium 12.4 mg/100 ml. The Bence Jones reaction in urine was negative. The remainder of the routine laboratory tests were normal. Cytology of the bone marrow smear revealed a replacement of normal cells by myeloma cells. The nucleus of many of these myeloma cells was centrally placed and showed large nucleoli. Immunofluorescent studies of these cells (performed by Miss H R E. Schult) showed the presence of a monoclonal IgA protein of the kappa type. The direct Coombs test was negative. The total serum protein was 10.4 g/100 ml. Agar and immunoelectrophoresis revealed an IgA paraprotein. Ultracentrifugation showed the presence of a large amount of IgA with sedimentation constants of 8.5, 10 and 12 Svedberg units (S). These protein components accounted for 69% of the total protein. Investigation of haemostatic functions showed several disturbances: see Table I.

Roentgenological examination of the skeleton showed no abnormalities. A diagnosis of multiple myeloma was made and treatment with blood transfusions, prednisone in a dose of 40 mg per day and alkeran in a dose of 5 mg per day was started. A total of 70 mg alkeran was administered. The clinical condition of the patient improved markedly within a month. The haemoglobin value had risen to 13.8 g/100 ml, serum calcium was 8.9 mg/100 ml. Ultracentrifugation demonstrated a decrease in IgA con-

chlorambucil was started the patient was free from symptoms for almost a year. A relation of the paraprotein to the bleeding does not seem likely since the level was always very low and the spontaneous decrease in 1964 was not attended by a clearcut improvement. It should be noted that factor VIII deficiency in macroglobulinaemia was also found by Nielehn (24) who could not show a direct effect of the paraproteins on this deficiency in his patients.

In case 4 the chlorambucil therapy seemed to have a favourable effect on the bleeding tendency, since the bleeding returned on several occasions when the drug was stopped or lowered. This phenomenon seemed unrelated to the presence of the paraprotein which was always at a very high level. It should be stressed however that a causal relationship between the therapy and the disappearance of bleeding cannot be proved in view of the irregular occurrence of such bleedings.

The clotting defects in Waldenström's macroglobulinaemia have been attributed to coating of platelets by the macroglobulin or to various effects on the vessel wall or on clotting factors (13, 18, 23, 24) although the observations of Perry (26) did not confirm the latter view.

In the myeloma case there was an influence of therapy on the haemostatic functions which seemed

to be related to the level of the paraprotein. Increased serum viscosity may have caused the bleeding tendency here but an effect of paraproteins on the clotting mechanism has also been postulated (7, 12, 32).

Viscosity

Bleeding tendency has been attributed to increased serum viscosity caused by the pathological macroglobulin (see Table II). In case 3 the viscosity was not different from that of normal serum while in case 4 the viscosity was consistently high as could be expected from the paraprotein level. Treatment with chlorambucil had no effect on viscosity in the latter case although the bleedings decreased considerably except in the retina. Mechanical considerations would lead one to assume that the retinal vessels are a very sensitive target organ for deviations caused by a high blood viscosity. Our observations in case 4 do not exclude this possibility which calls for further investigation. The serum viscosity was high before treatment and decreased afterwards in case 5. This case also dem-

onstrates that a strongly increased serum viscosity need not be caused by a macroglobulin of the IgM type so that viscosity measurements cannot always support the diagnosis of Waldenström's macroglobulinaemia.

General discussion

The study of our cases demonstrates that alkylating agents do not always have an effect on the paraprotein level although this has often been observed. Underdosage as a cause of failure to affect the paraprotein level seems to be unlikely because of the existence of drug-induced leucopenia in all patients. The clinical effect of therapy may be attributed to prednisone in cases 1 and 2 and cannot be conclusively demonstrated in cases 3 and 4. A clearcut correlation between paraprotein level and clinical symptoms seems to be absent in all cases.

The conflicting statements concerning the effect of the paraprotein on various symptoms of Waldenström's macroglobulinaemia may reflect the individuality of these abnormal proteins. Individual differences can be found in electrophoretic mobility, solubility and L or H chain type and offer the possibility to make monospecific antisera (16). These differences may also be found with regard to interference in clotting mechanism etc. and might also explain why for example an increased serum viscosity does not always result in a bleeding tendency (cf. 10). It is therefore very possible that in some cases the paraprotein level is indeed a useful parameter for following the course of the disease. This however has first to be established by prolonged observation of the individual case. Before this has been done neither serum viscosity nor paraprotein level are sure to give an indication of the clinical condition.

The single myeloma case taken for comparison in this study seems to confirm this rule since the most important symptom, the osteolysis, became visible when the paraprotein was at its lowest level. Alexanian et al. (1) however found the myeloma protein level to be an important parameter of disease activity in a study of 82 patients.

It may be inferred from our observations that the formation of an IgM paraprotein in Waldenström's macroglobulinaemia is a process not necessarily related to other symptoms of the disease. This means that the view of the paraprotein formation as the primary defect implicit in many dis-

sions may be questioned. The production of clonal IgM rather is one of many symptoms expressing themselves in varying degrees without the underlying cause being known at present.

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A COMPARATIVE STUDY CONCERNING THE STABILITY OF THE ANTICOAGULANT EFFECT OF ACENOCOUMAROL AND PHENPROCOUMON

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Abstract Two comparable groups, each of which comprised 41 patients, were treated with long acting phenprocoumon and short acting acenocoumarol, respectively for six months. When phenprocoumon was used, a significantly more stable anticoagulant effect was seen. This was reflected by a greater percentage of thrombotest values within the intended therapeutic zone ranging from 5 to 10% thrombotest activity inclusive. Moreover there was a difference in the facility with which patients could be kept in the therapeutic range, the number of alterations that had to be made in maintenance dose and control interval was less with phenprocoumon than with acenocoumarol.

The results obtained by long term treatment with anticoagulants to prevent cardiac infarction depend upon the intensity of this treatment (7-12). Also venous thromboses can be better prevented by an intensive treatment schedule (13). Though there are indications (2, 3, 5, 6, 11, 14, 15) that the long acting coumarin derivative phenprocoumon, will yield a more stable anticoagulant effect than other coumarin drugs, it is not known whether this will hold also if constantly low contents of vitamin K dependent factors are aimed at.

In this paper data are presented which were collected during intensive treatment with long acting phenprocoumon (Marcoumar[®]) and short acting acenocoumarol (Sintrom[®]) in two groups of patients during a period of six months. The thrombotest values obtained in all patients and the ease with which the values to be aimed at could be realized with each of the drugs under study were analysed statistically.

MATERIAL AND METHODS

Comparability of the two groups of subjects

A random selection was made of 110 subjects out of a group of patients who had been under prophylactic treat-

ment with anticoagulants for more than one year. These subjects were treated as outpatients with acenocoumarol. They were classified according to the variations observed in thrombotest values during eight months prior to the study. Thrombotest results were expressed as percentages of coagulation activity. The mean and the standard deviation (s.d.) of the percentages of coagulation activity were calculated for each patient. s.d. varied from $\pm 1.8\%$ in patients with stable hypocoagulability to $\pm 5.6\%$ in a very unstable patient. Because of these large differences in stability it was decided to make a paired comparison experiment. Fifty five couples were formed so that the difference in s.d. between partners was as small as possible. Beginning with January 1967 acenocoumarol was replaced by phenprocoumon for one of the partners in each of the 55 couples, the choice being made by drawing lots. The stability of the anticoagulant effect of the two drugs was compared in the period between April 1, 1967 and October 1, 1967. The period of comparison was not started before April 15 because the use of a new drug may at first influence the accuracy with which the prescribed dose is taken.

Twenty-six patients dropped out between the moment of patient selection and the end of the period of study. Two patients appeared to be wrongly classified, one of whom took phenprocoumon before the investigation, while the other was not an outpatient. Six patients died. Eight patients were taken to hospital for a complicating disease. In four patients a control was taken over by another thrombosis service. In three patients treatment was stopped by their doctor who was not the thrombosis service specialist. One patient appeared to be resistant to acenocoumarol.

Because both patients dropped out in only a few couples, four new couples were formed in which the difference in s.d. prior to the study was not larger than in the former ones. In total, 41 couples remained for comparison. Seven of these 87 patients have dropped out for a short time during the period of the investigation. In the remaining period, however, at least six thrombotest determinations were done. The group of patients who started taking phenprocoumon and the group of those who went on taking acenocoumarol were in close conformity with each other. Mean ages were 57 and 60.6 years, respectively.

Table III Dosage scheme

IT activity	Control interval	Dose
In therapeutic range 5-10	3 weeks	Unchanged
<5	≤1 week	1 or 2 days nil then changed or unchanged
≥11 to ≤15 following 2 controls in therapeutic range	3 weeks	Unchanged
>15* following 2 controls in therapeutic range	<3 weeks	Unchanged
* of 3 subsequent control ≥11	<3 weeks	Changed

RESULTS

The stability of the anticoagulant effect of acenocoumarol and phenprocoumon in two groups of 41 patients each

After the formation of pairs of comparable patients and the substitution of phenprocoumon therapy for the acenocoumarol treatment in one of the partners of each pair (January 1967 until April 15 1967) a comparison was made between the thrombotest values obtained in each of two partners during the period from April 15 to October 15 1967. In the period of comparison 364 thrombotest determinations were made in the phenprocoumon group and 394 in the acenocoumarol group.

The stability of the anticoagulant effect can be assessed by calculation of the standard deviation of the thrombotest percentages of each patient during the period of comparison. There was a high degree of stability ($s.d. \leq 3$) in 31 of 41 patients taking phenprocoumon but only in 19 of 41 patients taking acenocoumarol. An unstable anticoagulant effect ($s.d. \geq 4.6$) was seen three times with phenprocoumon and 11 times with acenocoumarol (Fig. 1).

In the phenprocoumon group 78.3% of thrombotest activities were found to fall within the therapeutic zone aimed at ranging from 5 to 10% inclusive. This figure was 62.4% in the acenocoumarol group. A closer analysis of thrombotest values falling outside the therapeutic range was in favour of phenprocoumon (Table IV).

When a check was made as to the number of patients in whom all thrombotest results fell into the therapeutic range aimed at this appeared to be the case in 11 of 41 subjects treated with phenprocoumon and only in one of 41 subjects treated with acenocoumarol. A very poor result of anticoagulant therapy with less than 50% of thrombotests lying within the therapeutic optimum

envisaged was seen only in nine patients of the acenocoumarol group (Fig. 2).

If stability of adjustment in a group of patients treated with coumarin drugs is not satisfactory the number of controls and the number of times the dose has to be changed will be greater than if a constant hypocoagulability has been achieved. The number of times the dose had to be changed was 116 in the acenocoumarol group as compared with 58 in the phenprocoumon group (Table V).

The dosage range of a patient i.e. the difference between the maximum and minimum dose prescribed during the period of comparison was on the average 0.3 mg when phenprocoumon was used as compared with 0.7 mg for acenocoumarol. These averages have been corrected for the difference in action potency: 4 mg acenocoumarol corresponding to 2.7 mg phenprocoumon (Table VI).

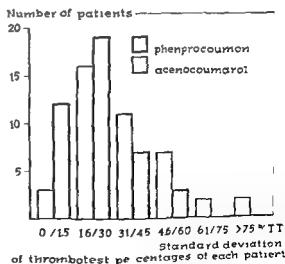


Fig. 1 Stability of the anticoagulant effect when using phenprocoumon and acenocoumarol for six months

Table I Comparability of the two patient groups in clinical respects

	Pats changing to phenprocoumon	Pats continuing to use acenocoumarol
No of males	34	33
No of females	7	8
Average age	58.7	60.6
Indication for treatment		
Coronary insufficiency or infarction	27	32
Claudication or vascular graft	9	4
Various indications	5	5
Heart failure	5	7
Total no of pats	41	41

the sex ratio was nearly identical and so were the indications for treatment and the frequency of heart failure (Table I)

As the pairs were formed on account of the stability of drug action during the months preceding the period of study an almost identical composition of the two patient groups with respect to the number of patients with stable and unstable levels of hypocoagulability the dose of acenocoumarol administered and the number of blood controls required could be expected and was in fact found (Table II)

Control of the anticoagulant effect

The effect of the coumarin derivatives used on blood coagulation was measured by means of the thrombotest using the prescription proposed by the manufacturer (Nyegaard & Co Oslo Norway) In the morning

venous blood was taken in disposable polypropylene tubes with 3.8% trisodium citrate dihydrate as an anticoagulant in a ratio of 9 parts of blood to 1 part of citrate solution. The thrombotest determination was made within 5 hours after taking blood. Determinations were made at least once every three weeks in each patient

Dosage and application of coumarin derivatives

The dosage of phenprocoumon 3 (1-phenylpropyl)-4-hydroxycoumarin (Marcoumar®) 3 mg per tablet, and acenocoumarol 3 (alpha-acetoxyl-p-nitrobenzyl)-4-hydroxycoumarin (Suntrom®) 4 mg per tablet was adjusted by one of us (W B) according to the scheme shown in Table III. Patients were advised to take all of the dose prescribed at one time in the morning. Beginning with January 1967 a thrombotest activity ranging from 5 to 11% was aimed at which was lower than in the preceding period.

Table II Comparability of the two patient groups as concerns the effect of anticoagulant administration in the period preceding the period of comparison (Jan 1 1966 to Sept 1 1966)

	Pats changing to phenprocoumon	Pats continuing to use acenocoumarol
Standard deviation of TT activities in a pat		
Average	5.6	5.6
Range	1.8-12.3	1.8-12.6
No of pats with		
100% of controls between 6 and 20 TT activity	15	13
80-100% of controls between 6 and 20 TT activity	13	14
<80% of controls between 6 and 20 TT activity	13	14
Dose of acenocoumarol (mg/day)		
Average	3.27	3.17
Range	1.0-6.0	1.5-5.4
Difference between maximum and minimum dose/pat		
Average	0.64	0.6
No of controls/pat		
Average	11.2	10.5
Range	7-29	8-19

Table VI Dosage and dosage range (difference between maximum and minimum dose per patient) using phenprocoumon and acenocoumarol

	Use of phenprocoumon	Use of acenocoumarol
mean dose/day (mg)	2.7	4.0
mean dosage range in mg (difference between maximum and minimum dose/pat.)	0.2	0.7
mean "corrected" dosage range in mg (correction for anti-coagulant activity 4 IU mg acenocoumarol = 2.7 mg phenprocoumon)	0.3	0.7

and were excluded from the statistical analysis if the differences between the members of each pair were smaller than 5%. In 25 of the remaining 35 pairs the percentage of controls within the therapeutic range was in favour of the phenprocoumon treated patients. This difference proved to be significant ($p = 0.0007$) (Table VII). An advantage of phenprocoumon partners was found in 17 of 30 pairs for percentages of TT values $\geq 15\%$, in 23 of 34 pairs for percentages $\geq 11\%$ and in 16 of 22 pairs for percentages $< 5\%$. All these differences were significant (Table VII).

3. *Dosage range and number of controls* After exclusion of six couples in which one or both of the partners were treated for less than six months and of the pairs showing small differences the phenprocoumon patients showed significantly

smaller dosage ranges and less dose alterations than their acenocoumarol partners (Table VII).

Complications and toxicity

Despite the intensity of hypocoagulability during 479 months on therapy there were only 5 small bleedings and for these no specific treatment was required. Because of the rarity of bleeding events a comparison of this risk during treatment with acenocoumarol and phenprocoumon was not feasible. No toxic side effects were seen.

DISCUSSION

It had already been demonstrated by Borchgrevink (1) that an intensive treatment with oral anticoagulants in patients with angina pectoris reduces

Table VII Significance of differences found in the stability of the anticoagulant effect and the ease with which this was attained in pairs of patients using phenprocoumon and acenocoumarol respectively

	Total no. of couples compared	No. of couples		Undecided	p-value
		In favour of phenprocoumon treated partner	In favour of acenocoumarol treated partner		
Standard deviation of the percentages of TT activity/pat.	41	27	6	8	0.002
Percentages of TT values/pat. within the therapeutic range	41	25	10	6	0.0007
Percentage of TT values/pat. outside the therapeutic range					
Percentage $\geq 15\%$ TT activity	41	17	3	21	0.003
Percentage $\geq 11\%$ TT activity	41	23	11	7	0.007
Percentage $< 5\%$ TT activity	41	16	8	19	0.03
Corrected dosage range	35	27	5	3	0.002
No. of dose alterations	15	24	5	6	0.001
No. of controls	35	18	10	7	0.07

5 couples of which at least one partner was treated for less than six months were excluded.

Differences considered to be too small.

p-value (observed level of significance) of Wilcoxon's two-sided signed rank test.

mortality and frequency of infarctions if this group is compared with patients on a less intensive treatment schedule. According to Rozenberg et al (12) the occurrence of arterial thromboses was higher in patients with a desirable therapeutic range of 10–20% thrombotest activity than in patients treated more intensively. A long term anticoagulant treatment after myocardial infarction effecting a profound and stable hypocoagulability (between 5 and 11% thrombotest activity) yielded favourable clinical results according to Loeliger et al (7) and Meuwissen et al (8). If such an intense and stable hypocoagulability is aimed at without increasing the bleeding hazard too much, a meticulous control of the patients and of the anticoagulant activity of the coumarin drug to be used is inevitable (4, 9, 10).

Although a more constant effect might be expected from the long acting drug phenprocoumon, no data about the quantitative differences between this drug and acenocoumarol were available. Nor was it known whether differences might remain if patients were treated intensively. The differences found in this study are distinctly in favour of the long acting coumarin drug phenprocoumon.

The use of the standard deviation of the percent ages of thrombotest activity can be criticized because thrombotest values lower than 5% cannot be determined exactly. However in practice the standard deviation turned out to be a quite suitable measure of the stability of the treatment for individual patients and in this study the evaluation of the experimental results using other measures of stability like the percentage of TT values within the therapeutic range completely confirmed the conclusions based on the use of the standard deviation.

The greater instability found with the use of acenocoumarol is almost certainly not due to a more frequent use of other drugs that might have interfered with the coumarin action. Information obtained from the patients has given the impression that drug consumption in both groups was almost equal.

Generic and trade names of drugs

Phenprocoumon Marcoumar, Liquamar, Falntron.
Acenocoumarol, Sintrom, Nicoumalone.

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MONOCLONAL GAMMOPATHY (IgG) AND CHRONIC ULCERATIVE DERMATITIS (PHAGEDENIC PYODERMA)

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Abstract Case report of a patient with chronic ulcerative dermatitis and monoclonal gammopathy. No signs of myeloma could be detected. The M-component was found to be of class G L-chain type K and subtype 3.

served a female patient with chronic ulcerative dermatitis (phagedenic pyoderma) whose serum likewise contained an IgG M component.

CASE REPORT

Patient P G a woman aged 60. In the summer of 1966 this patient developed a painful ulcerative lesion of the left lower leg, which was refractory to conventional therapeutic measures. A similar lesion subsequently developed on the right lower leg. At examination an ulcerative abnormality was found on the dorsal aspect of the left and the ventral aspect of the right lower leg. There were no superficial varices. The condition was identified as superficially localized ulcerative dermatitis with phagadenic dissemination and formation of numerous small pustules at many sites along the slightly elevated margin. The centre of the lesion showed coarse lamellar desquamation and crust formation, discontinuous at some sites (Fig. 1).

No abnormalities other than these skin changes were found.

Laboratory findings. ESR 80 mm after 1 hour. Haemogram normal. Urine normal. Normal blood sugar level. Liver function tests and electrolyte, creatinine and urea concentrations. At paper electrophoresis of the serum a homogeneous protein fraction was demonstrated in the medium speed γ -globulin region. This was confirmed by agar electrophoresis (Fig. 2a). Immunoelectrophoresis (with horse anti-human serum) showed that the M-component was of class IgG (Figs. 2b, c). With specific antisera the L-chains were of type K and the κ -chains (tested in immunoelectrophoresis and immunodiffusion experiments) of subtype γ 3 (Figs. 2c, d, e). Immunoelectrophoretic experiments showed diminished values for normal immunoglobulins IgG, IgA and IgM.

The finding of this M-component prompted an exhaustive study to establish the presence of multiple myeloma. Skeletal X-rays disclosed no abnormalities apart from some mild arthritic deformations. Repeated examination of the bone marrow failed to reveal any abnormality (specifically no plasmacytosis). No Bence Jones protein was

Monoclonal gammopathy is usually found in multiple myeloma (in which case the protein fraction consists of molecules of class IgG, IgA or IgD) and in Waldenström's macroglobulinaemia (in which case an M-component of the IgM class is present). M-components are being reported however with increasing frequency in various other diseases also (1, 3, 4, 5, 9). Skin diseases too can be associated with such a pathological protein fraction (6). In 1964 Rockl et al. (7) described six patients with chronic ulcerative dermatitis in four of whom an M-component of the IgA class was found. One of these four patients was suspected to suffer from multiple myeloma. Van der Sluis (8) described two patients and Meyers et al. (6) one patient with chronic ulcerative dermatitis and an IgA M-component. Danon et al. (1) reported on four patients without specifying either M-component classification or clinical details. There would seem to be a correlation between two rather infrequently occurring conditions, namely ulcerative dermatitis and monoclonal gammopathy of the IgA class. Degos et al. (2) recently described a patient with this skin disease whose serum contained an IgG M-component. So far as we know this is the first and only patient described in the literature who showed the combination of chronic ulcerative dermatitis and IgG paraproteinaemia. We observed

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THE GROWTH HORMONE DEPENDENT SULFATION FACTOR IN SERUM FROM PATIENTS WITH VARIOUS TYPES OF DIABETES

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Abstract The growth hormone dependent sulfation factor is low in serum from diabetics. There is no difference between the values obtained from obese and non-obese diabetics. Nor is there any difference between the values obtained from recently diagnosed untreated cases and long term diabetics with severe diabetic angiopathy. In non obese untreated diabetics a negative correlation obtains between the fasting serum sulfation factor and the serum glucose. One hour after ingestion of glucose the sulfation factor falls. In obese diabetics no correlation was observed between serum sulfation factor and serum glucose.

In a series of publications beginning in 1924 Houssay and coworkers demonstrated the importance of the pituitary gland for carbohydrate metabolism. They showed that hypophysectomized animals became extremely sensitive to insulin (15) that hypophysectomy alleviates the severity of pancreatectomy-diabetes (16) and that injection of pituitary extracts to partially pancreatectomized dogs induces a transitory diabetic state (17). In 1937 Young (38) working with intact dogs showed that injection of pituitary extracts repeated for a period of time resulted in a permanent diabetic state. In 1949 Cotes et al (8) produced a diabetic condition in the adult intact cat by repeated injections of purified ox growth hormone thereby proving that the diabetes inducing agent in the pituitary extract was growth hormone. These animal experiments have been confirmed in man by Luft (23). However their subjects treated with human growth hormone exhibited only a mild symptomless diabetes except for a group of hypophysectomized juvenile diabetics who developed a severe diabetic acidosis.

Growth hormone has been thought to play a role in the pathogenesis of human diabetes mellitus.

Young suggested in 1939 (39) that a short period of spontaneous pituitary hyperactivity could induce a stimulation of the beta-cells which eventually would lead to exhaustion of the beta-cells and a clinical diabetes mellitus.

There is however still not very much data in the current literature on which to estimate the significance of growth hormone in the pathogenesis of diabetes mellitus.

Serum growth hormone can be determined by the classical tibia test method although the sensitivity of this procedure is quite low or by Berson and Yalow's radio-immunological technique. In addition the growth hormone dependent Sulfation Factor (SF) can be determined by the method of Daughaday et al (10). This factor stimulates the uptake of radioactive sulfate in the costal cartilage of hypophysectomized rats. It decreases after hypophysectomy and increases after injection of growth hormone (3, 10, 36).

The few publications dealing with the problem of serum growth hormone in diabetic patients have employed one or the other of these methods (12, 13, 19, 32, 34).

The following is a report of a study of serum SF in a large group of diabetics. A comparative study of serum SF and immunological serum growth hormone as well as studies of immunological serum growth hormone during glucose loading will be published elsewhere (36, 37).

MATERIAL

The series of patients studied was a random group of 60 newly-diagnosed, untreated diabetics admitted to the Second University Clinic of Internal Medicine Aarhus Kommunehospital. Twenty seven were obese and 33 non-obese. Obesity was defined as a weight $> 115\%$ ideal

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Table I Average values of serum SF \pm SEM in non obese controls and various types of diabetes mellitus

	No of pats	Sulfation factor (mean \pm SEM)	Weight ()
	33 diabetics	0.78 \pm 0.03	<115
	27 diabetics	0.80 \pm 0.02	\geq 115
A	60 diabetics	0.79 \pm 0.02	—
B	22 diabetics (proliferative retinopathy)	0.73 \pm 0.04	—
C	12 non diabetics	0.98 \pm 0.05	<115

A < C $p < 0.001$ B < C $p < 0.001$

weight based on Hafslua Life Insurance Table (30). Average male and female weights in the age group 30–34 years in this table served as ideal weight. The table however refers to fully dressed individuals whereas the subjects included in the present material were weighed without clothes; consequently "ideal weight" was reduced by 1.5 kg and 1.0 kg for male and female subjects respectively.

The material also included 22 long term diabetics with severe diabetic angiopathy. These patients had not received insulin therapy for 24 hours before blood samples were taken.

The control group consisted of 12 subjects of normal weight with a normal fasting blood sugar and without fasting glucosuria.

The age of the diabetic patients varied between 12 and years; the same for the controls was 21–37 years of

age. Serum SF and glucose were determined in serum obtained from fasting subjects. In seven of the non-obese diabetics glucose loading (100 g glucose orally) was performed. Serum glucose and serum SF were determined in the fasting state and one hour after glucose. The degree of diabetes varied in the individual patients but all had a fasting blood sugar above 120 mg per 100 ml.

METHODS

All blood samples were drawn from a cubital vein at 8–9 a.m. after an overnight fast and rest, before the subject got out of bed. Immediately after venepuncture the blood was kept at +4°C for an hour and then spun at room temperature for 5 min at 3000 rpm in an ordinary laboratory centrifuge. The serum was stored at –20°C until analysis was performed.

Serum SF was determined by a modification of the original SF method as described in an earlier paper (35). In this technique costal cartilage was used from nonhypophysectomized young rats after a two-day fast. The index of precision obtained by using this technique is of the same order of magnitude as that described by Almqvist (2) employing Dasgupta et al's method (10) with a few modifications. One unit (U) of SF activity is defined as the activity of one ml of the pooled reference serum (35).

Serum glucose was determined by the glucoseoxidase method (18).

RESULTS

SF in serum from fasting subjects

Table I shows the average values in obese and non-obese newly diagnosed diabetics in patients with proliferative retinopathy as well as in lean non-diabetics. It appears that the average serum SF is considerably lower in the three diabetic groups than in the non-diabetics; this difference is statistically highly significant. Between the individual groups of diabetics however no difference could be demonstrated. Nor could any sex difference be demonstrated in the different diabetic groups.

The relationship between serum SF and serum glucose in newly diagnosed diabetics is shown in Fig. 1 (non-obese) and Fig. 2 (obese). As seen from Fig. 1 a negative correlation between serum SF and serum glucose can be demonstrated for the non-obese diabetics; serum SF being lower at higher blood sugar levels. The regression coefficient is –111.05 and the difference of the slope from zero is statistically significant ($p < 0.05$). Fig. 2 shows that no such correlation is found in obese diabetics.

Effect of administration of glucose on serum SF

Fig. 3 shows the serum SF values in the fasting state and one hour after glucose loading in seven newly diagnosed non-obese untreated diabetics. After glucose the serum SF fell in all cases although in some the fall was very pronounced and in others only slight. The mean decrease of serum SF was 27.5%, which is statistically significant ($p < 0.05$).

DISCUSSION

The nature of the sulfation factor in serum is not known. Physiological amounts of growth hormone added *in vitro* to the incubation mixture have no stimulatory effect on the sulfate uptake of isolated rat cartilage (2, 28). The stability of the serum sulfation factor is not identical with that of immunologically detectable growth hormone. Serum sulfation factor activity is stable for at least one week at room temperature (2, 7) while the amount of immunodetectable plasma growth hormone declines when plasma is placed for more than 24 hours at room temperature (33). Serum sulfation factor is thus not identical with immuno-

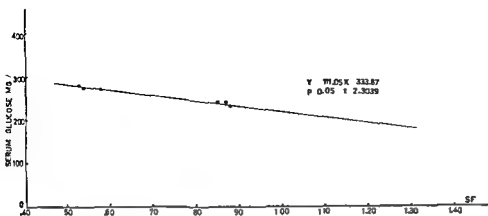


Fig 1 Relation between serum SF and serum glucose in 33 newly diagnosed untreated non-obese diabetics.

detectable growth hormone Daughaday et al (10) and Almqvist (1) have shown that the sulfation factor in serum from hypopituitary patients is low and that it increases after the patient has been treated with growth hormone injections. It has also been shown that acromegalic patients have elevated values of SF (9, 10, 20, 22). These results indicate that the serum sulfation factor is growth hormone dependent.

One of the results of the present study is the demonstration of a clear-cut reduction in serum SF in diabetic patients.

It is known that various other substances influence the uptake of radioactive sulfate in costal cartilage. Insulin in high concentrations (1–10 mU per ml) has been demonstrated to promote sulfate uptake (27, 29). Insulin concentrations of this order of magnitude have not been described in

normal subjects or diabetic patients and could thus not account for the differences obtained between our groups of diabetics and non-diabetics.

Salmon and Daughaday (29) found that the omission of one of the following amino acids—valine, threonine, isoleucine or leucine—from the incubation medium resulted in a decreased sulfate uptake in costal cartilage. Our incubation medium contains no amino acids except for those supplied by the serum to be assayed. There is however no evidence of highly abnormal plasma concentrations of these amino acids in diabetics (Carlsen et al

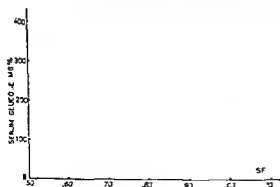


Fig 2 Relation between serum SF and serum glucose in 7 newly diagnosed untreated obese diabetics.

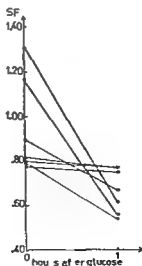


Fig 3 Serum SF levels in newly diagnosed untreated non-obese diabetics before and one hour after a glucose load (100 g).

(6) found only minor differences between diabetics and non diabetics in the serum concentration of amino acids. In the diabetics the concentration of valine, isoleucine and leucine was slightly higher while that of threonine was slightly lower than in the non-diabetic group.

The glucose concentration of the medium during incubation does not influence sulfate uptake. Salmon (27) found no difference in the uptake when the incubation medium contained 0 or 200 mg glucose per 100 ml.

The age range of the diabetic patients and of the control group in the present series is not identical; however, Almqvist and Rune and Daughaday et al (4, 10) found that serum SF was not influenced by age in adult subjects.

A high serum sulfate level in the serum will of course tend to lower serum SF by isotopic dilution. However, there is no reason to suspect sulfate retention in our patients, all of whom had a serum creatinine of less than 1.5 mg per 100 ml.

Corticosteroids and estrogens are known to inhibit the sulfate uptake in costal cartilage (11, 25). Plasma cortisol is known to be normal in uncomplicated diabetics (26, 31). Plasma estrogen values are not available in diabetics but as an indirect measure of this Horstmann (14) and later Jak (24) found a normal urinary excretion of estrogenic substances in large groups of diabetics.

As a whole it seems therefore that the low value of serum SF found in the diabetic patients included in the present study must be accepted as an expression of an abnormality which is characteristic of the diabetic state.

There are only a few studies of serum SF in diabetic patients. Almqvist et al (3) determined serum SF in two long term diabetics and found values of 1.19 and 0.64. Jensen et al (21) compared serum SF in obese and non obese newly diagnosed untreated diabetics. As in the present study no difference was observed. Their material did not include non-diabetics. Chesley et al (7) found a low serum sulfation activity in six pregnant diabetics compared to ten pregnant non diabetics.

It appears from the results obtained in the present study that there is a negative correlation between serum SF and serum glucose in a large group of untreated non-obese diabetics. It was also shown that when glucose was given to that type

of diabetic a fall in serum SF occurred in all cases. It is interesting to note that no correlation obtains between fasting serum glucose and immunodetectable growth hormone (37). On the other hand the fall in serum SF one hour after glucose is paralleled by the fall in immunological serum growth hormone (5, 19, 37).

From the studies of the response of immunodetectable plasma growth hormone to glucose in obese diabetics (5) it is known that these obese patients are either "normal responders", "poor responders" or "non responders". Thus the characteristic pattern of growth hormone response to a glucose load is a fall followed by a rise 4-6 hours later, may be either normal, absent or less pronounced than in normals. Our group of diabetics may well include all these three types. It is possible that a weak correlation between serum SF and serum glucose may be obscured by the heterogeneity of the group examined.

The low SF in fasting serum from diabetics cannot be explained today. Although serum SF is obviously related to growth hormone production in the pituitary gland, there is no correlation between serum SF and immunologically determinable serum growth hormone (36) and serum growth hormone determined in this way is not low in diabetes mellitus (37).

The only clinical condition in which serum SF is known to be reduced is in hypopituitarism; however, there is no evidence of reduced anterior pituitary function in diabetes mellitus.

It appears from the results obtained in the present study that in non-obese patients the reduction of serum SF is most conspicuous in patients with a high fasting blood sugar. Such a correlation was not demonstrable in the obese whose fasting blood sugar was on the whole somewhat lower as was to be expected. It would be of interest to see if it were possible to normalize the fasting serum SF by a strict normalization of the blood sugar level for a period of time.

It has been shown that serum SF falls after glucose. One hour after ingestion of 100 g of glucose when the blood sugar is high the serum SF values were usually distinctly lower than before. This finding is in accordance with the lower fasting serum SF values in patients with high fasting blood sugar but the course of the serum SF throughout the day and during the night is not known and a high blood sugar after glucose

may not be comparable to a high fasting blood sugar

It is interesting to note that serum SF is low after glucose at a time when immunological detectable growth hormone is known to be falling

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HETERO ANTIBODY AGAINST BILE CANALICULI IN PATIENTS WITH CHRONIC CLINICALLY ACTIVE HEPATITIS

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Abstract Antibody to the bile canaliculi of bovine liver was demonstrated in the serum from five patients with chronic clinically active hepatitis, in one out of ten patients with systemic lupus erythematosus and in one out of thirty one patients with positive antinuclear factor who did not suffer from systemic lupus erythematosus. The antibody was not detected in the serum from five patients with acute hepatitis eleven patients with cholecystitis and thirty five donors. The antibody was heterologous since it could not be demonstrated to bile canaliculi in normal human liver tissue nor to bile canaliculi in the liver tissue from two patients with chronic clinically active hepatitis. The antibody seems to be specifically directed against the bile canaliculi and it was always found concurrently with antinuclear factor against normal human liver tissue. Furthermore in the five patients suffering from chronic clinically active hepatitis the bile canaliculi antibody was found together with smooth muscle antibody and glomerulus antibody.

In the serum of patients with liver cirrhosis active chronic hepatitis and so-called lupoid hepatitis antibodies to cell nuclei (7-8) cytoplasm (9-12) and smooth muscle (4-13) have been found. In cases of lupoid hepatitis and active chronic hepatitis antibodies to renal glomeruli have been demonstrated (13). These antibodies are not species specific but react with both animal and human tissue and furthermore the cytoplasmic antibody is not organ specific. In patients with biliary cirrhosis virus hepatitis and post necrotic cirrhosis Paronetto (10) demonstrated antibodies to biliary ductular cells in the hepatic tissue from patients with active post necrotic cirrhosis or biliary cirrhosis but not to normal hepatic tissue.

The purpose of this report is to present the finding of an organ specific heterologous antibody to bile canaliculi in bovine liver found in the serum of patients with chronic clinically active hepatitis possibly complicated by liver cirrhosis.

METHOD

The indirect method of Coons was used for the demonstration of antibodies to antigens in tissue sections (1). Tissue frozen in liquid oxygen immediately after death or biopsy and stored at -20°C was sectioned at $6\ \mu$ in a cryostat. The unfixed sections were air-dried and then incubated first with inactivated serum and secondly with fluorescein-conjugated anti-human globulin for 30 min in a moist chamber at room temperature. After each period of incubation the sections were washed for 3 periods of 5 min in isotonic phosphate buffered saline at pH 7.2. Finally the sections were covered with phosphate buffered glycerol and glass slides. The reading was made in a Zeiss fluorescence microscope with an Osram HBO 00 W mercury lamp.

All the sera were first tested against bovine liver. All tests were made both with undiluted serum and serum diluted 1/10. Only if the sera showed a positive reaction at a dilution of 1/10 were the results considered to be positive.

MATERIAL

Serum was taken from five patients with chronic clinically active hepatitis, possibly complicated by cirrhosis, five patients with acute hepatitis, two patients with systemic lupus erythematosus (SLE) with liver affection, eight patients with SLE without liver affection, eleven patients with cholecystitis and thirty five blood donors. The five patients with chronic clinically active hepatitis had been ill for more than six months. Their clinical and laboratory findings are shown in Table I. In three patients, A II, L M and A M the diagnosis was verified through biopsy. Two patients, L M and A M had signs of cirrhosis. One patient, I B was not available for biopsy. In another patient O C biopsy could not be performed because of a constantly decreased prothrombin time. In the patients with active hepatitis the illness appeared after less than two months.

All the ten patients with SLE fulfilled seven of the following ten criteria for SLE, slightly modified after Jessar et al (3): 1 erythematous skin lesions, constitutional symptoms of cachexia, fever and weight loss.

Table I. Clinical and laboratory findings in five patients with chronic clinically active hepatitis

Pat	Sex	Age	Ascites	Hepato- megalia	Serum bilirubin (mg/100 ml)	Thymol turbidity test	Serum albumin (g/100 ml)	Serum gamma globulin (g/100 ml)	Serum alkaline phosphatase	Prothrom- bin (of normal)	Serum glutamic oxaloacetic transaminase (U/l)
I B	♀	46	-	-	2.3	0.71	2.9	4.6	16.9 KA	0	146
A H	♀	36	-	-	6.0	0.59	3.0	3.0	23.5 KA	68	177*
L M	♂	13	-	+	4.4	0.87	2.5	6.5	121 U/l	7	64
A M	♀	66	-	-	3.5	0.72	3.5	3.0	109 U/l	54	700*
O C	♀	33	+	-	6.5	0.50	2.5	3.7	275 U/l	16	10

Normal 2-11 King-Armstrong units

* Normal 3-12 U/l

Normal 22-64 U/l

3 absence of sepsis 4 arthralgia 5 renal disease 6 suppression of blood forming elements with leucopenia anaemia and thrombocytopenia 7 hypertrophy of lymph nodes liver or spleen 8 endocarditis 9 serous membrane effusion—pericardial pleural and peritoneal and 10 greater incidence in females Two of the patients with SLE had findings compatible with liver affection both had a positive thymol turbidity test and elevated serum glutamic oxalacetic acid transaminase and alkaline phosphatase One of the latter had furthermore hepatomegaly decreased serum albumin and elevated serum gamma globulin All the ten patients with SLE had a positive antinuclear factor (ANF) against cell nuclei of bovine liver None of the five patients with chronic clinically active hepatitis fulfilled the criteria for SLE as stated above

Furthermore the study comprised thirty-one patients who did not suffer from SLE, but who had a positive ANF against cell nuclei of bovine liver found at routine examination The majority of these patients suffered from rheumatoid arthritis They were not examined for liver diseases

RESULTS

As shown in Table II antibody to bile canaliculi of bovine liver was detected in all the five patients

Table II. Antibodies against bile canaliculi demonstrated by the indirect immunofluorescent technique

Diagnosis	No.	Bile canaliculi antibody
Chronic, clinically active hepatitis	5	5
Acute hepatitis	5	0
SLE with liver involvement	2	0
SLE without liver involvement	8	1
Cholecystitis	11	0
Blood donors	35	0
Diseases with positive ANF excl SLE	31	1
Total number	92	7

with chronic clinically active hepatitis in one patient with SLE without liver affection and in one patient with a positive ANF who did not suffer from SLE This patient had rheumatoid arthritis A positive reaction is presented in Fig 1



Fig 1. Immunofluorescent staining of bile canaliculi in fresh frozen bovine liver treated with inactivated serum from a patient with chronic clinically active hepatitis and fluorescein-conjugated anti-human globulin. Original magnification $\times 500$.

In most of the sera the positive reaction could only be demonstrated in the diluted specimen because of a non specific fluorescence in the undiluted specimen. None of the remaining sera contained factor reacting to bile canaliculi. Fig. 2 shows a negative reaction.

All sera containing bile canaliculi antibody were then tested employing the same technique and in the same dilutions for the possible presence of bile canaliculi antibody to normal human liver tissue and liver removed by biopsy from two patients with chronic clinically active hepatitis (L. M. and A. M.) for the presence of antibody to smooth muscle in the stomach of mice and blood vessels in normal human liver tissue for antibody to glomeruli in mouse kidney and cytoplasmic antibody to parietal cells in mouse stomach and to tubuli in mouse kidney.

It appears from Table III that none of the sera which were found to react with bile canaliculi of bovine liver reacted with bile canaliculi in normal human liver or liver specimens from the two patients whose sera reacted with bile canaliculi of bovine liver. All seven sera were also tested for the presence of ANF to the nuclei of bovine liver and normal human liver tissue. Six of them contained ANF to the nuclei in both bovine liver and normal human liver tissue whereas the serum



Fig. 2 No staining of bile canaliculi in bovine liver treated with unactivated normal human serum and fluorescein-conjugated anti human globulin. Original magnification $\times 500$.

Table III. Distribution of antibodies against bile canaliculi, nuclei, smooth muscles and renal glomeruli in serum from seven patients with heterologous bile canaliculi antibody.

Pat.	Diagnosis	Antigens								
		Bile canaliculi in				Nuclei in		Smooth muscles in		Glomeruli in
		Bovine liver	Normal human liver	Liver biopsy from L. M.	Liver biopsy from A. M.	Bovine liver	Normal human liver	Mouse stomach	Artery of human liver	Mouse kidney
I. B.	Chron. clinically active hepatitis	+	-	-	-	+	+	+	+	+
A. H.	Chronic clinically active hepatitis	+	-	-	-	+	+	+	+	+
L. M.	Chronic clinically active hepatitis	+	-	-	-	+	+	+	+	+
A. M.	Chronic clinically active hepatitis	+	-	-	-	-	+	+	+	+
O. C.	Chronic clinically active hepatitis	+	-	-	-	+	+	+	+	+
A. B.	SLE wth liver involvement	+	-	-	-	+	+	-	-	-
A. L.	Rheumatoid arthritis	+	-	-	-	+	+	-	-	-
No.		7	0	0	0	7	5	5	5	5

Positive only in undiluted serum.

Table I Clinical and laboratory findings in five patients with chronic clinically active hepatitis

Patient	Sex	Age	Ascites	Hepato- megaly	Serum bilirubin (mg/100 ml)	Thymol turbidity test	Serum albumin (g/100 ml)	Serum gamma globulin (g/100 ml)	Serum alkaline phosphatase	Prothrom- bin (of normal)	Serum glutamic oxalacetic transaminase (U/l)
I B	♀	46	—	—	2.3	0.71	2.9	4.6	16.9 K.A.	0	148*
A H	♀	56	—	—	6.0	0.59	3.0	3.0	23.5 K.A.	68	277
L M	♂	13	—	+	4.4	0.87	2.5	6.5	121 U/l	7	704
A M	♀	66	—	—	3.5	0.72	3.5	3.0	109 U/l	54	700
O C	♀	33	+	—	6.5	0.50	2.5	3.7	275 U/l	16	190

Normal 2-11 King-Armstrong units

* Normal 3-12 U/l

Normal 22-64 U/l

3 absence of sepsis 4 arthralgia 5 renal disease 6 suppression of blood forming elements with leucopenia, anaemia and thrombocytopenia 7 hypertrophy of lymph nodes liver or spleen 8 endocarditis 9 serous membrane effusion—pericardial pleural and peritoneal and 10 greater incidence in females. Two of the patients with SLE had findings compatible with liver affection both had a positive thymol turbidity test and elevated serum glutamic oxalacetic acid transaminase and alkaline phosphatase. One of the latter had furthermore hepatomegaly decreased serum albumin and elevated serum gamma globulin. All the ten patients with SLE had a positive antinuclear factor (ANF) against cell nuclei of bovine liver. None of the five patients with chronic clinically active hepatitis fulfilled the criteria for SLE as stated above.

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Chronic clinically active hepatitis	5	5
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Cholecystitis	11	0
Blood donors	35	0
Diseases with positive ANF but SLE	31	1
Total number	97	7

with chronic clinically active hepatitis in one patient with SLE without liver affection and in one patient with a positive ANF who did not suffer from SLE. This patient had rheumatoid arthritis. A positive reaction is presented in Fig 1.



Fig 1 Immunofluorescent staining of bile canaliculi in fresh frozen bovine liver treated with inactivated serum from a patient with chronic, clinically active hepatitis and fluorescein-conjugated anti-human globulin. Original magnification $\times 500$.

SIGNS OF URINARY TRACT INFECTION IN A HEALTH SURVEY OF FEMALE HOSPITAL EMPLOYEES

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Abstract A health survey has been made of urinary tract infection in 1346 female hospital employees. Pyuria in sediment of freshly voided urine was noted in 20% and found to be an unspecific symptom of urinary-tract infection. Significant bacteriuria was recorded in 2.5% and pyelonephritis as defined was diagnosed in 1.6%.

Pyuria and bacteriuria were more common in divorced and widowed women than in the others. Women who had borne children had a higher frequency of pyuria than nulliparae. Pyelonephritis was diagnosed more frequently in women over 30 years old. A difference in the frequency of pyuria was noted between occupational groups and also between the personnel of a large somatic hospital and the personnel of a large mental hospital.

This paper reports studies of the frequency of bacteriuria and other signs of urinary tract infection in female hospital personnel employed by Malmöhus County Council. In addition to studies of the frequency of pathological findings indicating urinary tract infection the investigation also included various factors such as age, civil status, parity, occupation and place of work which might play a part in the pathogenesis.

MATERIAL

In connection with a gynaecological health survey of County council hospital personnel at Lund the urine of 1346 women between 15 and 69 years old was examined by screening. 696 of the investigated women were also asked to complete a questionnaire concerning mainly urinary-tract diseases. Because of pathological findings in the urine and/or histories suggestive of urinary tract disease 644 women were examined clinically. Among the screened women 843 worked in a large general hospital (Lund's Lasarett), 383 in a large mental hospital (St. Lars) and 73 in small institutions, such as nursing homes, homes for the aged, and children's homes.

METHODS

A Screening

Urine specimens were collected before the gynaecological examination which was made between 7 and 8 o'clock in the morning. The women were instructed to void into sterile vessels after careful vulval cleansing with compresses soaked with sterile saline solution. The urine specimens were then immediately cooled to about +4°C.

All the specimens were examined by the following procedures:

1 **Quantitative urine culture** Some of the specimens were transferred to a sterile tube (cooled to about +4°C) and sent to the hospital's bacteriological laboratory (Head Prof. R. Grubb) for semiquantitative urine culture. The bacterial count was quantitated according to the scale: no growth, less than 10^2 , 10^{2-4} and more than 10^4 bacteria per ml of urine.

Sediment Ten ml of well mixed urine were centrifuged for 5 min at 3000 rpm. The supernatant was decanted away so that 3 or 4 drops remained. The sediment was shaken and 1 drop of urine was examined under the cover glass at magnification $\times 370$.

3 **Tests for proteinuria** Albustix according to the maker's instruction and precipitation with sulphosalicylic acid (0%).

4 **Uroscreen**, the triphenyl tetrazolium-chloride (TTC) test (3) Hemastix and Clinstix.

B Clinical examination

The women whose urine showed any of the following abnormalities were examined clinically: more than 10^4 bacteria per ml of urine, more than 5 white cells per high power field, more than 5 red cells per high power field, high bacterial counts in the sediment, Albustix readings of ++ or higher, positive sulphosalicylic acid test, glycosuria, positive Uroscreen or TTC test, positive Hemastix or Clinstix.

635 (47%) of the total number of 1346 women underwent the clinical examination which took place within 1 to 8 weeks after the screening. All the aforementioned analyses were repeated at the clinical examination and in addition the 1-hour excretion of formed elements in

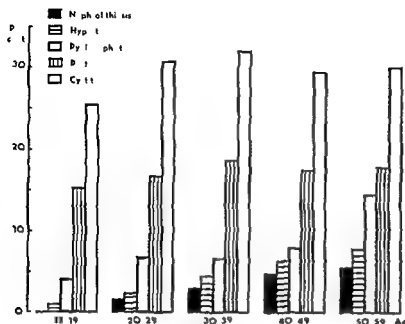


Fig. 1. Frequency of nephrolithiasis, hypertension, pyelonephritis, proteinuria and cystitis in the history of the 696 women who completed the questionnaire at the screening examination.

the specimens was calculated by Addis count and specific gravity of the urine was determined after 20 hours of water deprivation. The sampling technique was the same as that used at the screening but the specimens were usually collected later in the day. The clinical examination included a careful history and a medical examination.

In addition to the aforementioned analyses the following laboratory procedures were also carried out: determination of Hb, ESR, antistreptolysin titre and serum creatinine.

A radiological investigation, including plain X-ray of the urinary tract and intravenous pyelography was made on special indications in 257 women.

C. Definitions

(a) *Bacteriuria*. A bacterial count of more than 10^5 organisms per ml of urine in one specimen denoted *bacteriuria*; a count of 10^{4-5} organisms per ml of urine denoted *suspected bacteriuria*; a count of more than 10^5 organisms per ml of urine in two consecutive cultures with identical bacterial flora denoted *significant bacteriuria*.

(b) *Pyuria*. More than 5 WBC/HPT or more than 4 mill WBC/12 h.

(c) *Pyelonephritis*. In accordance with Orsten (39) we considered that pyelonephritis was present when the case fulfilled at least three of the following criteria:

1. A history suggestive of pyelonephritis.
2. Significant bacteriuria.
3. Impaired maximal concentration capacity (specific gravity <1.025, urine volume <300 ml per 12 hours).
4. X-ray findings interpreted as evidence of pyelonephritis or an anatomical change with an increased tendency to pyelonephritis (urinary tract malformations, nephrolithiasis, and hydronephrosis).
5. Pyuria (more than 4 mill WBC/12 h).

(d) *Suspected pyelonephritis or urinary-tract infection*.

When two of the said criteria were present, the condition was classed as suspected pyelonephritis or urinary tract infection.

D. Grouping of the investigated women

In the analysis of the results the women were grouped by age, civil status, parity, occupation, and place of work. With respect to occupation, they were divided into three categories: A, B, and C. A = nurses and their equals; B = auxiliary nursing personnel and their equals; C = a mixed group comprising senior student nurses, office staff and others.

RESULTS

A. A History of Renal Disease

Fig. 1 shows the frequency of previous urinary tract disease and high blood pressure according to the questionnaire.

1. *Cystitis*. Altogether 209 or 30% of the 696 women had had cystitis. The frequency was independent of age, civil status, occupation and place of work.

2. *Pyelonephritis*. 53 women or 7.6% had had pyelonephritis. The frequency was significantly higher for the women over 49 years old ($p < 0.05$ (Fig. 1)). With respect to civil status, parity, occupation and place of work, there were no significant deviations.

3. *Nephrolithiasis*. 19 or 2.7% of the women said that they had had renal stones earlier.

4. *Hypertension*. 28 or 4.0% of the women

Table I. Frequency of urinary tract diseases in the history and urinary findings in the screened women in relation to age, civil status, parity, occupation and place of work.

Screening examination	History					Laboratory findings				
	No of women examined	Cystitis (%)	Pyelonephritis (%)	Nephrolithiasis (%)	Hypertension (%)	No of women examined	Pyuria WBC/HPF (%)	Pyuria WBC/HPF (%)	Bacteriuria organisms/ml (%)	Bacteriuria organisms/ml (%)
							> 5	> 10	≥ 10 ³	> 10 ⁵
Age (y)										
15-19	99	25.5	4.1	—	1.0	131	18.0	4.9	4.6	2.3
20-29	255	30.8	6.8	1.6	2.4	460	18.6	10.0	11.7	4.6
30-39	123	32.1	6.7	3.0	4.5	260	19.5	10.4	8.4	5.8
40-49	126	29.6	8.0	4.8	6.4	253	0.9	10.9	9.6	5
50-59	90	30.3	14.6	5.6	7.9	237	21.0	11.7	12.6	5.6
Civil status										
Unmarried	325	27.4	5.8	2.2	0.9	555	17.7	7.0	7.4	3.1
Married	314	31.8	8.0	6.1	5.1	688	17.9	9.7	11.6	5.8
Divorced	31	35.5	19.4	6.5	6.5	45	24.4	15.6	17.8	13.3
Widowed	15	33.3	16.7	6.7	13.3	32	28.1	21.9	9.4	3.1
Parity										
0-para	353	30.0	6.5	2.8	2.0	705	17.6	7.1	8.9	3.5
1-para	121	31.4	9.9	4.1	5.8	234	17.5	11.1	9.8	4.3
2-para	129	34.9	7.8	7.0	3.9	231	18.1	9.1	9.5	6.1
≥ 3-para	90	23.3	8.9	5.6	4.4	172	23.3	14.5	14.5	8.7
Occupational category										
A	331	28.7	9.1	2.9	1.9	441	12.2	6.5	9.6	4.7
B	195	34.9	7.7	0.8	5.6	481	2.6	11.6	8.7	4.8
C	137	28.5	5.1	0.5	2.9	367	20.7	9.3	12.3	5.2
Place of work										
University hospital	175	30.9	8.0	7.4	7.4	843	20.0	10.3	9.4	5.0
Municipal hospital	381	28.1	8.1	3.7	1.8	383	12.8	7.0	10.7	4.7
Others	73	34.2	5.5	1.4	2.7	73	19.2	4.1	12.3	2.7
Total	696	30.3	7.6	2.7	4.0	1346	19.6	10.0	10.3	4.9

had a history of high blood pressure. The frequency of renal stone and high blood pressure increased as a percentage but not significantly with age.

B. Laboratory Findings at the Screening

I. *Pyuria defined as more than 5 WBC/HPF* was noted in 244 (19.6%) of 1346 investigated women. The results are summarized in Table I.

Age, civil status, parity. The frequency was largely the same in the different age groups. As regards civil status there were no significant relations but the frequency of pyuria was higher as a percentage for divorced and widowed women than for the others (26.0% as against 17.8%). Nor was there any significant variation for parity but the percentage was higher for women who had borne three or more children than for the others (23.3% as against 17.7%). See Table I.

Occupation. The frequency of pyuria as a percentage was higher in categories B (auxiliary nursing personnel) and C (mixed group) than in category A (nurses). The difference was significant in the total material between categories B (22.6%) and C (20.7%) on the one hand and category A (12.3%) on the other ($p < 0.0005$ and $p < 0.005$ respectively) and in the age groups 20-29, 40-49 and 50-59 between categories B and A ($p < 0.025$, $p < 0.01$ and $p < 0.025$ respectively).

Place of work. The frequency of pyuria was higher in the general hospital than in the mental hospital in all age groups except the lowest (in total 20.0% as against 12.8%). The difference was significant for the whole series and for the age groups 30-39 and 40-49 ($p < 0.005$, $p < 0.05$ and $p < 0.025$ respectively). In order to exclude the possibility that the difference in frequency between the places of work was due to different

distribution of occupational categories a comparison was made between the same categories of personnel at the two hospitals. It was found that both in category A and in category II the frequency was higher in the general hospital for the age groups above 20 years. The difference was significant for the age group 30-69 years ($p < 0.005$ and $p < 0.05$ respectively).

2 *Pyuria defined as more than 10 WBC/HPF* was noted in 125 (10.0%) of 1346 women whose urine was examined with respect to sediment.

Age There was no significant variation with age but the frequency in the age group 15-19 years was lower than in the others (4.9% as against 10.0-11.7%).

Civil status The frequency was significantly higher for the divorced and widowed women than for the rest in the total series ($p < 0.01$). There was no significant difference between married and unmarried women.

Parity The frequency was throughout lower for nulliparae than for women who had borne children and the difference was significant for the total series (7.1% as against 11.3%, $p < 0.01$).

Occupation As regards the frequency in different occupational categories the result was the same as that for pyuria defined as more than 5 white cells per high power field that is a higher frequency for most of the age groups in categories B and C than in category A. The figures in the respective age groups were too low for statistical treatment but in the total series there was a significant preponderance in category II in comparison with category A ($p < 0.01$).

Place of work Similarly to the result for pyuria defined as more than 5 white cells per high power field the frequency was higher in all the age groups in the general hospital but the difference was not significant. When a comparison was made between the occupational categories the result was still the same the difference being significant for category A in the age group 30-69 ($p < 0.05$).

3 Quantitative urine culture

(a) *10³ or more bacteria per ml of urine* 135 (10.3%) of 1346 women had 10³ or more bacteria per ml of urine.

Age The frequency was lower in the women under 20 years old than in the higher ages ($p < 0.05$). No other variation with age was noted.

Civil status The frequency was significantly higher both for married and for divorced women than for unmarried women ($p < 0.025$ and $p < 0.05$ respectively).

Parity The frequency was significantly higher for women who had borne three or more children than for the nulliparae (14.5% as against 8.9%, $p < 0.05$). No other distinct tendencies were noted.

Occupation Significant differences between the occupational groups were noted only in the age group 20-29 years in which the frequency was significantly higher in category C than in categories B and A (19.7% as against 8.8% and 10.1% respectively, $p < 0.05$ in both cases) and in the age group 30-39 years in which the frequency was higher for auxiliary nursing personnel than for nurses (16.2% as against 5.4%, $p < 0.05$).

Place of work There was no correlation between frequency and place of work.

(b) *More than 10 bacteria per ml of urine* 66 (4.9%) of 1346 women had more than 10 bacteria per ml of urine.

Age Similarly to the results under (a) the frequency was lower in the lowest age group than in the others (2.3% as against 4.6-5.8%) the difference was not significant.

Civil status The frequency was significantly higher for married and for divorced women than for unmarried women (5.8% and 13.3% respectively as against 3.1%, $p < 0.05$ and $p < 0.005$ respectively).

Parity As regards parity a tendency similar to that for women with 10³ or more bacteria per ml of urine was noted that is a higher frequency for women who had borne two or more children than for nulliparae (7.2% as against 3.5%, $p < 0.025$).

Occupation place of work There was no noteworthy difference between the occupational groups or between places of work.

C Clinical Examination

635 out of the 1346 women who underwent the screening tests attended the clinical examination. This group did not differ significantly from the primary material with respect to age, civil status, parity, occupation or place of work.

1 Pyelonephritis

Pyelonephritis according to the definition used in this study was diagnosed in 21 (3.3%) of the 635

Table II Frequency of pyuria bacteriuria suspected pyelo nephritis/urinary tract infection and pyelonephritis in the 635 clinically examined women in relation to age civil status parity occupation and place of work

	No of women examined	Quantitative urine culture				Suspected pyelo nephritis ()	Pyelo nephritis ()
		Addis count > 4 mill ()	WBC/12 h > 10 mill ()	> 10 ³ bact /ml ()	Significant bacteriuria ()		
Age							
15-19	55	36.4	14.5	3.6	3.6	5.5	1.8
20-29	245	34.7	15.5	4.5	2.4	4.5	0.8
30-39	115	27.8	13.9	10.4	9.6	16.5	1.7
40-49	114	33.3	19.3	6.1	6.1	7.9	4.4
50-69	106	35.8	25.5	8.5	4.7	19.8	9.4
Civil status							
Unmarried	266	33.5	15.8	5.6	3.4	7.5	1.8
Married	325	32.3	16.9	6.8	5.8	11.1	3.7
Divorced	25	32.0	32.0	8.0	8.0	20.0	12.0
Widowed	16	56.0	37.5	12.5	6.3	6.3	12.5
Parity							
0-para	321	32.2	15.8	5.3	3.7	7.4	2.2
1-para	109	36.7	17.6	3.7	8	9.2	5.5
2-para	117	33.3	17.9	8.5	5.1	10.3	1.7
≥3 para	86	34.9	19.8	11.6	11.6	18.6	7.0
Occupational category							
A	193	28.5	16.1	6.2	5.7	8.3	2.9
B	224	40.6	23.7	7.1	4.5	10.3	4.5
C	167	39.5	15.0	7.8	6.9	13.8	2.2
Place of work							
University hospital	433	34.6	18.2	6.5	4.6	9.2	3.0
St Lars hospital	170	27.0	14.1	5.9	5.3	11.8	4.1
Others	25	48.0	28.0	12.0	8.0	2.1	4.0
Total	635	33.4	17.5	6.5	4.9	9.9	3.3

women examined clinically (unless otherwise stated the percentages reported in the following are calculated on the number of clinically examined women). This means that pyelonephritis was diagnosed in 1.6% of the 1346 women who took part in the health survey.

As regards the criteria used for the diagnosis of pyelonephritis pyuria was present in all cases. Next in order was a history of pyelonephritis (14 cases) followed by radiographic changes (13 cases). Significant bacteriuria was present in seven women that is one third of the cases and in paired concentration capacity in six women.

Age It will be seen from Table II that the frequency of pyelonephritis was clearly higher in women over 39 years old than in younger women (7.3% as against 1.2% $p < 0.0005$).

Civil status The frequency was higher for divorced and widowed women than for the rest and higher for married than for unmarried women.

The figures were 12.2% for divorced and widowed, 3.7% for married and 1.8% for unmarried women. The figures were too low for statistical treatment.

Parity The frequency was higher for women who had borne three or more children than for women who had borne fewer or no children (7.0% as against 2.7% the figures were too small for statistical treatment).

Occupation The frequency was higher for category B than for categories A and C (4.5% as against 2.9% and 2.2% respectively the figures were too low for statistical treatment).

Place of work There was no noteworthy difference in frequency between places of work.

At the screening examination of the 21 women in whom pyelonephritis was diagnosed eight stated that they had had proteinuria. Two women had had cystitis, one had had hypertension and three had had renal stones.

The screening analysis of the urine showed Albustix⁺⁺ or more in two cases positive sulphosalicylic acid tests in six more than 5 WBC/HPF in eleven and 10³ or more bacteria per ml of urine also in eleven cases

2 *Suspected pyelonephritis or urinary tract infection* 62 (99%) of the 635 clinically examined women fulfilled two of the five afore mentioned criteria for a diagnosis of pyelonephritis. These cases comprised 4.7% of the total series

The most common finding was pyuria which was present in 48 (77%) of the 62 women. Second in order were significant bacteriuria and radiographic changes each present in 21 women (34%). Impaired concentration capacity was noted in 10 cases (16%) and 24 cases (39%) had a history of pyelonephritis

Age The percentage of women who fulfilled two criteria for pyelonephritis varied significantly with age ($p < 0.0005$). A significantly lower frequency was recorded for women under 30 years of age (4.5–5.5%) than for the higher age groups (7.9–19.8%). The frequency in the age group 40–49 years was significantly lower than in the age group 50–69 years (7.9% against 19.8% $p < 0.025$) and lower as a percentage but not significantly in the age group 30–39 years (16.5%)

Civil status occupation place of work The number of cases did not permit statistical calculation but as a percentage there was no distinct tendency with respect to the distribution by civil status occupational groups or places of work

Parity The frequency of suspected pyelonephritis increased as a percentage with increasing number of childbirths (from 7.4% for nulliparae to 18.6% in women who had borne three or more children). The frequency for women who had borne two or more children was significantly higher than for those who had borne one or no child ($p < 0.01$)

Seventeen (28%) of the 62 women with urinary tract infection or suspected pyelonephritis stated that they had had proteinuria and nine (15%) had had cystitis. Six (10%) stated that they had had hypertension and six (10%) had had renal stones. The urinalysis at the screening examination showed Albustix⁺⁺ or more in four (6%) positive sulphosalicylic acid test in seven (11%) more than 5 WBC/HPF in 32 (51%) and 10³ or more bacteria per ml of urine in 29 cases (46%)

If the above groups under 1 and 2 are combined it will be found that 84 out of the 1346 investigated women (or 6.3%) showed symptoms which according to our criteria indicated urinary tract infection or suspected or probable pyelonephritis

In the combined group of women who fulfilled two or more criteria for pyelonephritis there was the same relation to age civil status parity occupation and place of work as in the separate groups but in addition a significant difference was noted between auxiliary nursing personnel and nurses in the age group 20–39 years (8.4% against 2.4% $p < 0.01$)

3 *Quantitative sediment*

(a) *More than 4 mill WBC/12 h* (without history or other evidence of urinary tract infection) Out of the 635 clinically examined women 213 (33.5%) had pyuria alone

Age civil status parity The frequency of pyuria showed no noteworthy relations to age parity or civil status

Occupation The frequency of pyuria in the age groups above 19 years was higher in categories B and C than in category A. The difference between B and A in the age group 40–69 years was significant (44.8% as against 22.2% $p < 0.005$)

Place of work In the age groups above 19 years the frequency of pyuria was higher in the general hospital than in the mental hospital the difference being significant in the age group 50–69 years (48% as against 14.3% $p < 0.005$). The possibility cannot be excluded however that this difference was due to an overrepresentation of auxiliary nursing personnel among the examined women from the general hospital

(b) *More than 10 mill WBC/12 h* 111 (17.5%) of the 635 examined women had more than 10 mill white cells per 12 h without history or other evidence of urinary tract infection

Age There was no significant variation with age but the frequency as a percentage was higher in the age group 50–69 years than in the other age groups (25.5% as against 13.9–19.3%)

Civil status The frequency was significantly higher for divorced and widowed women than for married and for unmarried women (34.1% as against 16.9% and 15.8% respectively $p < 0.025$ and $p < 0.01$ respectively)

Purity No distinct tendency was noted with respect to the number of pregnancies and the frequency of pyuria.

Occupation In the age groups above 19 years the frequency was higher in category B than in categories A and C between which there was no difference. The difference was significant in the combined age group 20-69 years (for category B 25.7 for A 15.7% and for C 16.0% $p < 0.025$ and $p < 0.05$ respectively).

Place of work In the age groups above 19 years the frequency was higher in the general hospital than in the mental hospital. The differences were not significant and could possibly also be due to a dissimilarity in the number of examined women in the respective occupational groups from the two hospitals.

DISCUSSION

General Comments

The terms pyuria, bacteriuria, urinary tract infection and pyelonephritis have not been uniformly defined in the literature. With a technique of analysis similar to that used by us at the screening examination the borderline for pyuria ranged from 3 to 20 white cells per high power field (31). As regards leucocyturia in quantitative sediment there are also differences of opinion about the normal range (3, 19, 27).

As to the clinical significance it should be emphasized that pyuria as the only symptom need not be equivalent to urinary tract infection and that such infection can often occur without pyuria. This holds true for the assessment of single tests in particular. The relation between pyuria and bacteriuria varies from one published report to another (33).

In accordance with Kass (8, 9, 10, 11) definitions of significant bacteriuria the borderline between contamination and urinary tract infection has generally been set at 10 organisms per ml in two consecutive cultures with identical bacterial flora although opinions differ on this point as well (14).

The potential part played by significant bacteriuria in the development of pyelonephritis has been convincingly verified by several authors (6, 9, 10, 11, 13, 14, 20, 22, 37).

To overcome the difficulty of classifying different types of infection of the urinary tract, Freed

man (5) simply lumped all of them together under the term urinary tract infection.

Some uncertainty attaches to the definition of pyelonephritis chosen by us (according to Ørsten (39)) and overdiagnosis as well as underdiagnosis will undoubtedly occur in our series.

Because of the varying clinical definitions of pyelonephritis it is difficult to compare different published materials. At the Second International Symposium on Pyelonephritis Strauss (30) therefore advised every author who writes about urinary tract infections to define the term he uses.

Comments on our Results

The material presented must be regarded as selected since it comprises hospital personnel. In Sweden a certificate of health is required for employment in hospitals and this may mean that some persons with pyelonephritis without subjective symptoms will be rejected. On the other hand the possibility cannot be excluded that with the bacterially contaminated hospital environment the risk of infections of the urinary tract is also greater.

History. The assessment of historical data such as those relating to cystitis mostly diagnosed by the patients themselves is bound to be very uncertain. Psychogenic micturition disturbances and uncharacteristic dysuria are often interpreted by the patient as cystitis. On the other hand it is to be expected that the usually mild disorders caused by cystitis will soon be forgotten and this might explain the relatively small proportion (30%) of women with a history of cystitis in our series. For the sake of comparison it may be mentioned that Salimander and Øst (26) in a control series of non bacteriuric women found that 50% of them had a history of urinary tract infection. The figures for the frequency of pyelonephritis (7.6%) of renal stone (2.7%) and of high blood pressure (4.0%) seem to be more reliable since these diagnoses are usually based on data supplied by a physician.

Laboratory procedures. Published figures for the frequency of pyuria in non bacteriuric women show great variation probably owing to the aforementioned factors but the frequency in our series 19.6% is fairly close to the reported mean figure of 17.8% which one of us (33) found in a survey of the relevant literature.

Data on the frequency of asymptomatic bacteriuria also vary (6 8 19 23 35). Our figures for the frequency of bacteriuria (more than 10 organisms per ml of urine in one culture 4.9%, in two cultures 2.5%) agree well with the frequency reported by Kass (10) and by Sallmander and Öst (26) for subjectively healthy women namely 4.4% and 5.0% respectively. Our low figure for significant bacteriuria based on two consecutive cultures might be explained by the long interval between the cultures and by the fact that the second urine specimen represented day urine with short bladder incubation.

A diagnosis of pyelonephritis according to our criteria was established in only 21 (1.6%) of the 1346 women taking part in the health survey. If the women who had suspected pyelonephritis or urinary tract infection are added to the pyelonephritis group the frequency will be 6.3%.

The frequency of pyelonephritis as well as that of suspected pyelonephritis or urinary tract infection was significantly higher in the higher age groups over 29 years which is in agreement with general experience.

Most authors (9 11 17 23 26) have found that prevalence of asymptomatic bacteriuria increases with age but Vejlsøgaard (35) noted a peak increase only after the age of 70 in his series. As regards bacteriuria in pregnancy there is a difference of opinion about its relation to age. Some authors (6 7 11 25 32 37) found that the frequency rose with increasing age whereas others noted no age difference (2 38) or the highest frequency at the age of 20 (11.9% as against 8.3% at the age of 30 (16)). The age variation of asymptomatic bacteriuria has been attributed to various factors such as sexual activity pregnancies gynaecological disorders and social conditions. In a series of patients with symptomatic urinary tract infections Loudon and Greenshalgh (18) noted a peak in the age group 20-29 years then there was a fall followed by a second rise after the age of 70.

In the present series the frequency of cystitis in the history did not vary with age. On the other hand we noted as was expected that the frequency of pyuria showed no significant relation to age.

The higher frequency of bacteriuria in the women over 19 years old in our series could primarily be presumed to be due to the fact that a greater number of women older than 19 years are

married and have regular sexual intercourse. It is also possible that completed pregnancies would play a part.

As far as women are concerned marriage usually means changes in sexual habits and socioeconomic status. In addition the number of pregnancies is higher in married than in unmarried women. Therefore higher prevalence of urinary tract infections in the married would not be unexpected and has in fact been reported by several authors. Loudon and Greenshalgh (18) in their series of women with urinary tract infections found a higher frequency among married than among unmarried nulliparae. Sleight et al. (29) examined 397 women who visited an infertility clinic and found a frequency of bacteriuria of 8% whereas no case of bacteriuria was detected among 100 pupil midwives. The higher frequency among the infertile women may possibly be explained in part by the instrumental interventions they had undergone because of their infertility. Chalmers (2) found a frequency of bacteriuria of 5-6% in married nulliparae as against 1% in young unmarried nulliparae. The difference was presumed to be due to different sexual activity.

In our series the frequency of pyuria was higher in divorced and widowed women than in the rest both at the screening examination (more than 10 WBC/HPF) and at the clinical examination (more than 10 mill WBC/12 h). On the other hand there was no difference between unmarried and married women. The frequency of bacteriuria was significantly higher in divorced and widowed and in married women than in unmarried women (9.1% 5.8% and 3.1% respectively). The same tendency was noted for pyelonephritis although the difference was not significant (12.2% 3.7% and 1.8% respectively).

The high frequency of latent and manifest urinary tract infection during pregnancy has in recent years attracted increased interest and many comprehensive investigations have been made into the subject. The high prevalence has been attributed to the changes morphological and others which accompany pregnancy but it has also been suggested that increased sexual activity at the beginning of pregnancy would play a part. It would therefore be reasonable to presume that the prevalence of bacteriuria would increase with the number of completed pregnancies as it has in fact been

found to do in some studies of pregnant (2 22 25 32 34 37) and non pregnant women (21) Vejlsøgaard (35) and Williams et al (38) on the other hand noted no relation between bacteriuria and parity. Kass (9) found a more distinctly increased frequency only after seven pregnancies. Unlike these authors Forkman (4) and Petersdorff (24) noted a higher frequency in primigravidae than in multigravidae and Petersdorff concluded that there does not seem to be an increased rate of acquisition with parity.

In our series there was a higher frequency of findings indicating urinary tract infection in women who had borne children than in nulliparae which would support the view that completed pregnancies would increase the prevalence of urinary tract infection. The possibility cannot be excluded however that other factors such as sexual habits and social and hygienic conditions would play a more important part than pregnancy in itself.

The fact that social and economic conditions play a part in the causation of certain diseases has long been recognized. In spite of the socioeconomic equalization that characterizes the development in many countries in the 20th century the social factors must still be considered to be of aetiological significance. As regards urinary tract infection for instance the frequency has been found to vary between social groups living under different conditions. Kass (11) noted a higher frequency of bacteriuria in the agricultural population than in the urban population in Jamaica and Turck et al (34) in accordance with Whalley (36) Beard and Roberts (1) recorded a higher frequency in pregnant women of lower socioeconomic status. In this connection a parallel may be drawn to the observation that cervical cancer is more common in some socioeconomic groups than in others. Since our investigation was planned mainly as a purely somatic and not as a sociological study we were not able to group the women according to social and economic class. We therefore chose to make a rough division by occupation well aware of the fact that in Sweden a particular occupation has often no special relation to social and economic conditions. The investigation showed that pyuria was throughout more common among the auxiliary nursing personnel than among nurses and that there was no such difference for bacteriuria. It is possible that the higher frequency of

pyuria among the auxiliary nursing personnel could be explained by unsatisfactory collection of urine specimens owing to less medical experience. Another possibility which cannot be excluded is that the close contact that the women in this group have with the patients in their daily work might play a part.

The accumulation of infections in a general hospital and the modern intensive antibacterial treatment which has led to the development of pathogenic nosocomial bacterial strains should mean that the personnel in such a hospital are exposed to greater risk of infections. An interesting observation was made by Kennedy et al (12) who found that some serotypes of *E. coli* which produce diseases of the urinary tract were more common in the intestines of hospital inpatients than in outpatients. We therefore found it justifiable to compare the frequency of urinary tract infection among women working in a large general hospital with that in the personnel of a large mental hospital and small nursing institutions respectively. The comparison showed that pyuria was more common in the general hospital personnel than in the others also when the composition of the personnel in the different hospitals was taken into consideration and that there was no difference in frequency as regards bacteriuria. In view of the fact that pyuria as was mentioned in the foregoing is an unspecific sign of urinary tract infection the results do not justify the conclusion that the prevalence of urinary tract infections would be higher among the general hospital personnel.

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ARE PROTEINURIA TESTS RELIABLE AS SCREENING METHODS FOR RENAL DISEASE?

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Abstract To assess the value of proteinuria tests as screening method for the detection of urinary tract disease an evaluation has been made of the Albustix and the sulphosalicylic acid tests in a health survey of 1346 women between 15 and 69 years old, all of whom are employed in hospitals.

The study comprised screening of the total material (1346 women) and a clinical examination of 634 women with pathological urine findings at the screening and/or histories suggestive of urinary tract disease. The clinically examined women were tested with Albustix on four occasions and the sulphosalicylic acid test on three occasions. At the screening 3.5% were Albustix positive and 5.9% positive in the sulphosalicylic acid test. In 33.1% there was a discrepancy between the two tests. Of the clinically examined women 0.3% were Albustix positive on all four occasions, 2.4% on two or three and 47.2% on one occasion. The corresponding figures for women examined by the sulphosalicylic acid test on three occasions were 1.7% positive on all three occasions, 6.8% positive on two and 22.3% on one occasion. The frequency of intermittently positive proteinuria tests decreased significantly with increasing age. The frequency of urinary tract disease and hypertension was the same in the group with intermittently positive proteinuria tests as in the group with negative tests throughout. The results show that proteinuria tests are not suitable as the only screening method for the detection of urinary tract diseases.

In our clinical work we have often observed discrepancies between the results of proteinuria tests and other clinical findings. We have therefore undertaken to investigate the constancy of the Albustix results when the test was repeated on various occasions on the same subject during a health survey in women as well as its correlation to the sulphosalicylic acid test and other signs of renal disease. Possible influences of various parameters—age, marital status, parity, occupation and place of work—were also studied.

In another study the same material was analysed for the frequency of symptoms indicative of urinary tract infection (15).

MATERIAL AND METHODS

The material comprised 1346 women whose ages ranged between 15 and 69 years and who were employed at the County Council hospitals in Lund. The investigation, which was made in connection with a health survey included screening and clinical examination. Among the screened women 843 were working at a large general hospital (Lunds lasarett) 383 at a large mental hospital (St. Lars Sjukhus) and 73 at smaller institutions, such as nursing homes, homes for the aged, and children's homes.

The screening tests were made on fresh morning urine voided after careful vulval cleansing with sterile isotonic saline solution and comprised the following analyses:

1. *Albustix*. Urinary protein concentration estimated according to the manufacturer's grading. 2- to 4- were considered as positive reactions and 1- as intermediate.

2. *Precipitation with 10% sulphosalicylic acid*. Weak precipitation was assessed as intermediate (+) and distinct to marked precipitation as positive.

3. *Microscopy of sediment*. Pyuria was defined as more than 5 white cells per high power field and haematuria as more than 5 red cells per high power field.

The following methods were also applied: quantitative urine culture, the triphenyl tetrazolium-chloride (TTC) Clusfix, and Hemastix tests.

For technical reasons, only the later part (696 women) of the investigated material, which did not differ significantly in any way from the total material, were asked to complete a short questionnaire about renal and urinary tract diseases.

Those who had histories suggestive of renal disease or whose urines were abnormal in the screening tests were advised to appear for a clinical examination within 1 to 8 weeks. The 634 women who returned for this examination did not differ significantly from the screened women with respect to age, marital status, parity, occupation, and place of work.

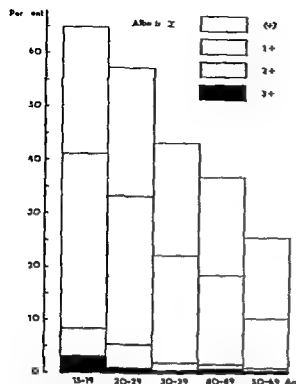


Fig 1 Albustix readings in different age-groups at the screening examination

The urinalyses included in the screening tests were treated at the clinical examination. The Albustix test was done on three and the sulphosalicylic acid test on two occasions during the same 4 hour period (on submitted specimen of night urine for Addis count on freshly passed morning urine and, only for Albustix, on urine voided later in the day). When considered indicated a more comprehensive investigation was made including intravenous urography.

Significant haematuria was recorded as present when both the screening and the clinical examination showed positive Hemastix readings.

The women were grouped by occupation into hospital nurses (441), auxiliary nursing personnel (481) and a mixed group (167) comprising senior student nurses and office staff and others.

RESULTS

A A History of Proteinuria and Hypertension

Proteinuria 120 (17.2%) of 696 women had had proteinuria earlier. The frequency was significantly higher in married than in unmarried women (20.7% and 13.5% respectively, $p < 0.025$). For those formerly married (divorced plus widowed) the frequency was 19.6%. A history of proteinuria was also more common among women who had borne children than among nulliparae (20.3% and

14.2% respectively, $p < 0.05$). There was no significant difference between married and unmarried women without children (12.7% and 16.5% respectively). The percentage of women with a history of proteinuria was higher among those working at the big hospital than among those in the small institutions (18.8% and 6.8% respectively, $p < 0.025$). There was no correlation between a history of proteinuria and occupation or age.

High blood pressure 28 women (4.0%) had a history of high blood pressure. The frequency had a tendency to increase with age though not significantly.

B Laboratory Findings at the Screening

1 Albustix

Frequency of positive results The urines of 47 (3.5%) of the 1346 women read 2+ to 4+ and those of 292 (21.7%) 1+.

Age (Fig 1) The frequency of Albustix (+) to 2+ fell gradually and significantly with increasing age ($p < 0.0005$).

Marital status, parity, occupation, place of work There were small percentage differences but without any special tendency and not significant, in the frequency of proteinuria in relation to each of these parameters.

Albustix-pyuria Table I shows the Albustix results in relation to the number of white cells per high power field in the sediment. It will be seen that the frequency of pyuria defined as more than 5 white cells per field was clearly higher in women with intermediate or positive Albustix readings than in those with negative readings (31.3% as against 15.8%, $p < 0.0005$). The frequency of pyuria was not significantly correlated with age.

Table I The results of the Albustix and the sulphosalicylic-acid tests at the screening examination compared with the frequency of pyuria on the same occasion

Results	Albustix		Sulphosalicylic acid test	
	Number examined	Sediment ≥ 5 WBC HPF (%)	Number examined	Sediment ≥ 5 WBC HPF (%)
Negative	953	15.8	710	13.8
Intermediate	259	30.5	183	9
Positive	41	36.6	37	37.7

7 Sulphosalicylic acid test

Frequency Out of a total of 978 investigated women 59 (5.9%) were positive and 187 (19.1%) doubtfully positive

Age (Fig 2) The percentage of positive sulphosalicylic acid tests showed no significant correlation with age. The percentage of doubtfully positive tests fell significantly with increasing age ($p < 0.0005$)

Marital status parity occupation place of work There were no noteworthy relations between a positive sulphosalicylic acid test and each of these parameters

Sulphosalicylic acid test—pyuria (Table I) Similarly to the Albustix results the frequency of pyuria was significantly higher in the women with doubtfully or clearly positive readings than in those with negative readings (30.1% as against 13.8% $p < 0.0005$)

3 Albustix-sulphosalicylic acid test

(Table II) It will be seen from the table that a total number of 978 women were examined by both the Albustix and the sulphosalicylic acid test. In 654 cases (66.9%) the results of the two tests were in agreement (580 59.3% negative 58 5.9% doubtfully positive and 16 1.6% positive) while in 324 (33.1%) there was a discrepancy between the two tests. Neither urinary pH nor pyuria could explain this discrepancy

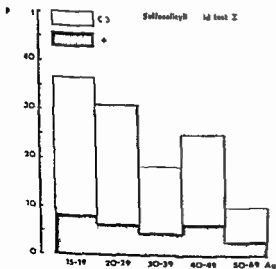


Fig 2 Results of the sulphosalicylic acid test in different age groups at the screening examination

Table II A comparison between the Albustix readings and the results of the sulphosalicylic acid test at the screening examination

Albustix readings	Sulphosalicylic acid test			
	Number examined	Negative ()	Intermediate ()	Positive ()
Negative 0 ₁ (+)	703	82.5	15.5	2.0
Intermediate +	231	6.8	25.1	12.1
Positive >2+	44	18.2	45.5	36.4

C Clinical Examination

1 Albustix

Frequency of positive Albustix (Table III) Forty-six (7.3%) of the 634 women who were clinically examined were positive (2+ to 4+) and 202 (31.9%) were doubtfully positive (1+) at the screening examination

In Albustix tests repeated on three occasions at the clinical examination 187 (48.4%) of the 386 women who were negative at the screening examination were negative throughout. Two (0.5%) were positive on all three occasions, two (0.5%) were positive on two occasions, and 35 (9.0%) were positive on one occasion. The rest (160) were doubtfully positive on one or more occasions.

The 202 women who were doubtfully positive in the screening test were negative in all the repeated tests except 24 (11.9%) who were positive on one occasion.

Of the 46 women who were positive at the screening examination 2 remained positive throughout, 1 positive in two repeat tests, and 10 positive in one repeat test. In the remaining 33 (71.7%) the first positive screening results could not be reproduced.

Accordingly of the 634 women who attended the clinical examination 2 (0.3%) had positive readings on all four occasions, including the screening test, and 3 (0.5%) in three tests out of four. In 12 (1.9%) the Albustix readings were positive twice and in 299 (47.2%) once.

History of proteinuria 147 (23.2%) of the clinically examined women had had proteinuria earlier. The proportion of those who had had proteinuria was significantly greater among those

Table III Results of the three Albustix tests at the clinical examination in the groups with negative intermediate and positive readings at the screening examination

0 = negative (+) = intermediate ++ = positive

Albustix screening	Number examined	Albustix readings in three tests at the clinical examination									
		000	00(+)	0(+)(+)	00+	0++	0(+)+	(+)(+)(+)	(+)(+)+	(+)(+)(+)	+++
		()	()	()	()	()	()	()	()	(*)	(*)
Negative	386	48.3	39.3	2.3	7.5	0.5	1.6	—	—	—	0.5
Intermediate	202	32.3	51.8	4.0	9.5	—	2.5	0.5	—	—	—
Positive	46	26.1	43.5	2.2	17.4	2.2	4.3	—	—	—	4.3

with positive Albustix readings on one or more occasions than among the rest (52.9% as against 22.4% $p < 0.01$)

Age It will be seen from Fig. 3 that the proportion of positive Albustix readings in one test decreased significantly with age ($p < 0.0005$) and that the proportion of positive readings in more than one test showed no special variation with age. The two women who were positive throughout are found in the age group 40–49 years.

Marital status The percentage of women in the age group 30–39 with one positive Albustix reading

was significantly higher among the married than among the unmarried women (32.4% as against 2.5% $p < 0.0005$). A tendency in the opposite direction though not significant was noted in the age group 20–29 (16.2% as against 25.0%).

Fertility In the age groups 20–39 the percentage of women with one positive Albustix reading was higher for those who had borne children than for the nulliparae (26.0% as against 19.5% and 11.4% as against 8.0% for the age groups 20–29 and 30–39 respectively). The differences were not significant.

Occupation No uniform tendency in the results was noted.

Place of work The proportion of women with one positive Albustix reading in the age group 20–29 was significantly higher for the mental hospital than for the somatic hospital group (36.1% as against 15.1% $p < 0.005$). The rest of the figures were too low for statistical analysis but no special tendency was noted.

Blood pressure In the age group 30–39 the mean diastolic pressure was significantly lower for the women with one positive Albustix reading than for those with negative readings throughout (70.0 mm Hg as against 75.6 mm Hg $p < 0.05$). The mean systolic pressure did not differ between the same categories of women. Thirty-two (5.0%) of the 634 investigated women had raised systolic pressure and 9 (1.4%) had raised diastolic pressure. One of the 34 women with hypertension was positive throughout and three were positive on one or two occasions.

Body weight, height and weight/height No significant differences were noted in any of these parameters between the women with one positive Albustix reading and those who were negative throughout.

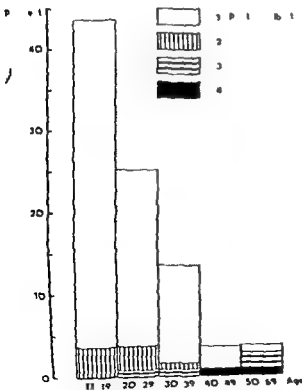


Fig. 3 Frequency of positive Albustix readings on 1, 2, 3 and 4 occasions in 634 women.

Table IV Frequency of the different clinical diagnoses in relation to the results of the four Albustix tests

Diagnosis	Albustix results					Total
	0000	000+	00++	0+++	++++	
Status post glomerulonephritidem	6	1	1	—	—	8
Hypertension	30	—	1	—	1	34
Chronic pyelonephritis	17	1	1	—	2	21
Chronic pyelonephritis?	24	1	—	—	—	25
Cystitis or urinary tract infection	33	9	2	—	—	42
Significant bacteriuria	25	6	—	—	—	31
Nephrolithiasis	3	1	—	—	—	4
Renal tumour	1	—	—	—	—	1
Urinary tract malformation	8	1	—	—	—	9
Number examined	525	92	12	3	2	634

Haematuria Twenty eight (4.4%) of the 634 clinically examined women had haematuria according to the definition given under Material. In three of these 28 the Albustix reading was positive once and in one it was positive in all four tests. The remaining 24 (86%) were throughout Albustix negative. The frequency of haematuria was higher in the age groups above 29 than in the age groups 15-29 (6.8% as against 1.7%, $p < 0.005$).

Diagnosis The diagnoses which were made by the examining physician after the investigation are recorded in Table IV. The two cases in which the Albustix readings were positive throughout were both diagnosed as chronic pyelonephritis. One of these patients had also hypertension. Of the three patients with three positive Albustix readings two were subjected to a more comprehensive nephrological investigation including renal biopsy which showed no abnormalities. The third who was investigated as an outpatient had a history of urinary tract infection but apart from proteinuria the clinical examination showed nothing abnormal.

In the group with negative Albustix readings

throughout the diagnosis was status post glomerulonephritidem in 8, hypertension in 26 and suspected or probable urinary tract infection or pyelonephritis in 72 cases.

In 4 of 21 cases diagnosed as pyelonephritis the Albustix readings were positive in one or more tests.

2 Sulphosalicylic acid test

Frequency of positive results (Table V) Of the 412 women who attended the clinical examination 48 (11.7%) had been positive in the sulphosalicylic acid test at the screening examination, 103 (25.0%) doubtfully positive and 261 (63.3%) negative.

Of the 48 women who were positive at the screening examination 14.6% were positive at the next two examinations while of the 261 women who were negative at the screening 19% were positive in both and 19.1% in one of the two tests made at the clinical examination. In the group with doubtfully positive results at the screening the frequency of positive results at the clinical examination was on the whole the same.

Table V Results of the two sulphosalicylic acid tests at the clinical examination in the groups with negative, intermediate and positive results at the screening examination

0 = negative (+) = intermediate ++ = positive

Sulphosalicylic acid test screening	Number examined	Results of the two sulphosalicylic acid tests at the clinical examination				
		00 ()	0(+) ()	(+)(+) ()	0+ ()	(+)+ (+)
Negative	261	58	0.7	—	18.4	0.7
Intermediate	103	44.7	7.8	2.3	19.4	2.9
Positive	48	9	0.8	—	39.6	14.6

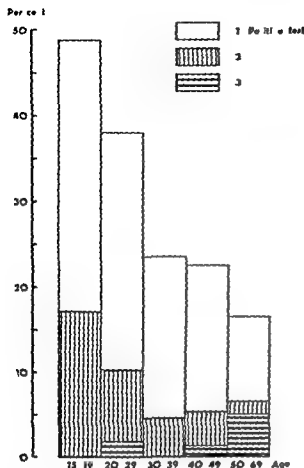


Fig 4 Frequency of positive sulphosalicylic acid tests on 1 and 3 occasions in 417 women

as in the group of women who were negative at the screening

Accordingly 7 women (1.7%) were positive on all three occasions (including the screening examination) 28 (6.8%) were positive on two occasions and 92 (22.3%) on one occasion whereas the remaining 285 (69.2%) were negative throughout

Age The frequency of intermittently positive cases fell significantly with increasing age ($p < 0.0005$) (Fig 4)

Diagnosis (Table VI) Two of the seven cases which were throughout positive in the sulphosalicylic acid test were diagnosed at the clinical examination as chronic pyelonephritis. One of these seven women had also hypertension. The other five showed no other evidence of renal or urinary tract disease. In the group with negative sulphosalicylic acid tests three cases were diagnosed as

status post glomerulonephritidem 13 as hypertension and 47 as suspected or probable urinary tract infection or pyelonephritis. Among the 11 cases assessed as chronic pyelonephritis the sulphosalicylic acid test was positive in 11 on one or more occasions

DISCUSSION

In order to assess the clinical value of tests for proteinuria we must first be able to define the criteria of proteinuria. But opinions differ on this point (10, 26, 35) probably because the investigations are made under different conditions on different materials and with the use of different methods for protein determination. The importance of the latter fact is illustrated by the discrepancy between the Albustix and the sulphosalicylic acid tests in the present study.

The Albustix test is a paper strip test based upon the protein error of tetrabromophenol blue. The strip is impregnated with the dye which is buffered to a pH of approximately 3.0-3.5. At this pH the amino groups of the urine proteins react with the dye and change the colour from yellow to green-blue. As albumin has relatively more amino groups than have globulins and mucoproteins, the kind of urinary proteins must be considered in the evaluation of the test. The Albustix test is for example not a reliable method for indicating β_2 -components in the urine (3, 10, 37).

Since it was first introduced (3, 6, 7) the test has been widely used both in clinical practice and in health surveys. Free after his studies credited the test with high specificity and sensitivity. But in later reports (33, 34) its

Table VI Frequency of the different clinical diagnoses in relation to the results of the three sulphosalicylic acid tests

Diagnosis	Results of the sulphosalicylic acid test					Total
	000	00+	0++	+++	++++	
Status post glomerulonephritidem	3	1	—	—	—	4
Hypertension	13	4	1	1	—	19
Chronic pyelonephritis	11	2	—	—	—	17
Chronic pyelonephritis in	14	3	1	—	—	18
Cystitis or urinary tract infection	—	13	3	—	—	16
Significant bacteriuria	9	9	6	—	—	24
Nephrolithiasis	3	1	1	—	—	5
Renal tumour	1	—	—	—	—	1
Urinary tract malformation	4	1	—	—	—	5
Number examined	290	9	3	—	—	417

value has been questioned, because it has been shown that factors other than the protein concentration influence the results of the test.

Even if it has been credited with greater reliability than the Albustix test the sulphosalicylic acid test also involves some sources of error. Turbid urine and the presence of mucoproteins may for instance cause false positive results.

The evaluation of a single test is also made difficult by the fact that even in normal persons the urinary excretion of protein will often increase during stress and physical activity (11, 27, 30). Moreover the concentration of the urine must also be taken into consideration. The time of sampling is therefore in general of significance in the evaluation of the result.

The high proportion of women with a history of proteinuria (17.2%) in our material should hardly be considered surprising since the investigated group had had numerous opportunities of having their urines examined for instance at health controls during and after school age examinations to obtain health certificates and pregnancy tests. As regards proteinuria in the history its clinical significance is difficult to assess one reason being as was mentioned above that different examiners use different criteria of proteinuria. Moreover in our series of women it was difficult to know whether the proteinuria had been intermittent or persistent.

The higher proportion of women with a history of proteinuria among those who had borne children as compared with the nulliparae (20.3% as against 14.2%) was expected not only because proteinuria is a common complication of pregnancy but also because pregnant women undergo careful testing of their urines. The marital status seems to be of no significance as the proportion was the same for married and unmarried nulliparae.

It is reasonable to presume that the personnel of a big hospital would have greater possibilities of being examined than would those working in small institutions and this is probably the explanation of the difference in the proportion of women with a history of proteinuria between these two groups although other causes cannot be excluded.

As far as we can find the literature contains no report of an investigation that is wholly comparable with the one presented here as among other factors our material can be regarded as selected (hospital personnel). Yet we found in the screening examination the same correlation between the frequency of proteinuria and age as that found by Thysell (1968 unpublished data)

in a public health survey and by others in investigations of men (32).

The results of the proteinuria tests obtained on the different occasions showed surprisingly poor agreement. Thus two of the 46 women who were Albustix positive in the screening examination were positive in the three subsequent tests whereas 33 were subsequently negative throughout. For the sulphosalicylic acid test the agreement seemed to be somewhat better.

Wolman (36) investigated 110 healthy physically active young men on eight occasions in the course of five days. 60% of them had proteinuria on one or more occasions, most of them (56%) were positive once only. In our selected material of women who were examined clinically we found that no less than 70.7% of the 634 women were doubtfully positive or positive and 17.2% clearly Albustix positive in at least one of the four urine tests for proteinuria. For the sulphosalicylic acid test which was performed on only three occasions similar figures were noted. Wolman's and our observations show how precarious it is to evaluate a single positive test for proteinuria.

The clinical examination showed that the higher frequency of proteinuria in the younger age groups noted at screening represented in the main intermittent or transient proteinuria. The genesis and the clinical significance of this form of proteinuria which has been described by various attributes (intermittent, physiological, transient, periodic, postural, orthostatic, orthotic, lordotic, cyclic, juvenile) have been the subject of many studies (3, 4, 8, 12, 13, 15, 16, 17, 19, 21, 27, 36). The increased excretion of protein has been ascribed to various causes for instance haemodynamic, endocrine and emotional factors.

Physical activity and stress can also cause increased protein excretion in healthy persons with resulting positive routine tests for proteinuria (19, 23, 27, 30, 36) and it is quite conceivable that greater physical activity in the younger age groups of a material like that presented here could explain in part the age variation for intermittent proteinuria. It is possible that a careful analysis of the single protein components could yield more detailed information (23, 24).

As mentioned above mucoproteins can give false positive proteinuria tests (6, 18, 33) and pyuria and contamination by vaginal secretion among other factors can therefore be sources of

error in a female patient material. We found indeed that the frequency of pyuria was significantly higher in the groups with intermediate and positive Albustix or sulphosalicylic acid tests at the screening examination but as it was not correlated with age this potential source of error could not have explained the age variation for positive protein tests. Theoretically the sulphosalicylic acid test should be more sensitive for mucoproteins than the Albustix test. We did not find any difference between the two tests, however with respect to pyuria.

The specimen can also give a false positive Albustix reading if the urine is alkaline and/or has a high buffer capacity. But in the present material there were no age variations in urinary pH which might account for the high proportion of positive Albustix tests in the lower age groups. The possibility that the urines of the younger women had a higher buffer capacity cannot be excluded, however. On the other hand the same correlation with age was found for the sulphosalicylic acid test as for the Albustix test.

King (13) in an investigation of young men with various forms of proteinuria noted diastolic hypertension (above 90 mm Hg) in 15% of the men with orthostatic proteinuria. No such tendency was noted in our material. We found instead that the diastolic pressure was significantly lower in the age group 30-39 of women with one positive Albustix test than with negative Albustix tests throughout.

A tendency to lower body weight has been noted in orthostatic proteinuria (4, 32). We found no difference in body weight, height or the ratio weight/height between the women who had intermittent proteinuria and those whose urines were throughout Albustix negative.

It has long been known (6) that microscopic haematuria may be present without proteinuria. This was verified in the present study in which we found that only four of 28 women with microscopic haematuria were Albustix positive on some occasion.

As regards the clinical significance of the intermittent proteinuria, opinions have changed. Earlier the condition was regarded as quite harmless but a more expectant and variable attitude has now been adopted. Follow up studies of large series of patients have shown that the intermittent proteinuria not seldom develops into a persistent form

and may sometimes represent a forerunner of renal disease. On examination of biopsy specimens organic renal changes have in fact been demonstrated in some cases (20, 22, 28, 29).

In some cases of asymptomatic intermittent proteinuria clinical investigations (3, 17, 36) biopsies (8, 20, 22, 28) or follow up studies (4, 5, 12, 16, 17, 32) have shown that this form of proteinuria may represent diverse pathological conditions such as pyelonephritis, glomerulonephritis, nephrolithiasis, hypertension, hypercalcaemia, hypopotasæmia, hyperuricaemia, diabetes, urinary tract malformations and the Fanconi syndrome. In our series the clinical assessment of intermittent proteinuria led to a clinical diagnosis (status post glomerulonephritidem, urinary tract infection, pyelonephritis, nephrolithiasis, hypertension and urinary tract malformations) in 23% (Albustix) and 28% (sulphosalicylic acid test) respectively while the rest of the investigated women with this kind of proteinuria were regarded as healthy.

As regards the persistent proteinuria its clinical significance has been considered to differ from that of the intermittent proteinuria (3, 5, 12, 13). The two women with positive Albustix readings throughout in the present study were clinically assessed as having chronic pyelonephritis. One of them had also hypertension. The same women were also positive in the three sulphosalicylic acid tests but there were five others with positive sulphosalicylic acid tests throughout who had no other signs of urinary tract disease.

It is well known that urinary tract disease can be present without demonstrable proteinuria, which was verified in the present study. We found that about one fifth of the women who had negative proteinuria tests throughout had some form of urinary tract disease or hypertension. Thus it seems that screening for proteinuria is not sufficient as the sole method for the detection of renal disease in health surveys.

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BLOOD LEVELS OF INSULIN IN URAEMIC PATIENTS

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Abstract Oral glucose tolerance and intravenous tolbutamide tests have been performed on 11 uraemic patient with normal fasting blood sugar on 9 non uraemic patients with normal oral glucose tolerance and on 13 non uraemic patients with abnormal glucose tolerance but with normal fasting blood sugar. At the same time the blood level of insulin was determined by an immunochemical technique. The glucose response of the uraemic patients during glucose tolerance as well as during tolbutamide tests did not differ from the average response in the group of non uraemics. On the other hand, the insulin response was increased in the uraemic patients during both tests. The uraemic patients also exhibited an increased insulin response to tolbutamide as compared with the 13 non uraemic patients with abnormal glucose tolerance.

Several authors have demonstrated impaired glucose tolerance by oral glucose tolerance tests in uraemic patients (5, 6, 10, 11). However in uraemic patients with a serum creatinine concentration exceeding 4 mg per 100 ml compared with control persons Andersen and Friis (3) found a significant difference in the two hour value only in patients below 60 years of age.

Attempts have been made also to elucidate the reaction to the tolbutamide test (14) in uraemic subjects. Ahlers et al (1) found an abnormal reaction whereas Westervelt and Schreiner (15) and Tchobrousky et al (13) reported normal findings. The latter authors found a normal insulin level in the blood of uraemic patients but a delayed increase during tests causing hyperglycaemia. According to criteria set up in a previous paper (2) Andersen and Friis (3) found a significantly increased number of abnormal tolbutamide tests in a series of uraemic patients when the serum creatinine exceeded 4 mg per 100 ml but only in patients under 60 years of age. Hampers et al (7) studied five patients with

severe uraemia before and after haemodialysis. They found reduced sensitivity to insulin in abnormal *iv* glucose test, abnormal tolbutamide test and reduced response of serum insulin to *iv* glucose. All these reactions returned to normal after haemodialysis. Briggs et al (4) have studied 12 uraemic patients and 14 controls. In their material too the oral glucose tolerance was reduced, the fall in blood sugar being delayed. Plasma insulin behaved much like the blood sugar showing also a delayed fall. Lastly Hutchings et al (9) studying 12 uraemic patients with a creatinine clearance below 4.8 ml/min found the glucose tolerance to be reduced in the majority while the insulin values increased more than in normal subjects during *iv* glucose tolerance tests.

PRESENT INVESTIGATIONS

Material

In order to assess the serum insulin concentration during oral glucose tolerance and intravenous tolbutamide tests in uraemic patients without diabetes and with a normal fasting blood sugar (<110 mg/100 ml) the following three groups of patients were investigated:

- A 11 uraemic patients with normal fasting blood sugar
- B 9 non uraemic patients with normal oral glucose tolerance
- C 13 non uraemic patients with abnormal glucose tolerance but with a normal fasting blood sugar

As uraemic patients we consider patients with a renal disease and a serum creatinine exceeding 1.5 mg/100 ml determined by autoanalyzer (Technicon).

The age distribution of the three groups was as follows:

- A 61.6 ± 8.4 y (43-78 y 7 over 60)
- B 48.5 ± 19.0 y (17-71 y 4 over 60)
- C 54.9 ± 18.7 y (19-85 y 6 over 60)

Table I. Clinical data for 11 patients with renal failure

Case no	Sex	Age	Diagnosis	Serum creatinine (mg/100 ml)	Serum urea (mg/100 ml)	BP (mm Hg)	Height (cm)	Weight (kg)
1	♀	61	Chronic pyelonephritis	15	65	160/100	167	61
2	♀	43	Chronic pyelonephritis	39	81	130/90	168	57
3	♀	64	Chronic pyelonephritis	40	62	150/90	156	42
4	♂	64	Chronic pyelonephritis	41	119	180/110	158	56
5	♀	69	Chronic pyelonephritis	42	90	150/95	157	61
6	♂	74	Chronic pyelonephritis	45		130/70	168	77
7	♂	63	Chronic pyelonephritis	47	111	180/100	180	97
8	♀	56	Chronic pyelonephritis	52	109	130/90	165	69
9	♀	70	Chronic pyelonephritis	57	92	135/70	159	57
10	♀	62	Chronic pyelonephritis	80	144	220/110	158	64
11	♂	53	Polycystic kidneys	156		140/100	156	47

There was no statistically significant difference between the age distribution in the three groups (between A and B $t=1.98$ $0.1>P>0.05$ and between A and C $t=0.73$ $P>0.1$).

No patient was obese.

The serum creatinine concentration in the uraemic patients was 15–156 mg/100 ml, mean 46.7 mg/100 ml. Nine had a concentration exceeding 40 mg/100 ml. All but one were normotensive and all had chronic pyelonephritis except one who had polycystic kidneys.

Methods

Glucose tolerance as well as tolbutamide tests were performed in the morning after fasting overnight.

The glucose tolerance test consisted in the administration of 1 g anhydrous glucose per kg body weight, the maximum dose being 70 g. Ear blood was drawn for

determining the blood level of glucose and venous blood for determining the serum insulin immediately before 15, 30, 60, 90, 120, 150 and 180 min after the intake of glucose. The tolerance test was considered normal if the 2-hour blood glucose level was less than 130 mg/100 ml (cf. Andersen and Frus (2)).

The tolbutamide test was performed by intravenous injection of 1 g sodium tolbutamide in the course of 1 min. Ear blood was drawn for glucose determination and venous blood for insulin determination immediately before and 10, 20, 30, 60 and 90 min after the injection. Moreover, venous blood was drawn 5 min after the injection. According to the criteria set up by Andersen and Frus (7) the response was considered normal when the 30 min blood glucose concentration was less than 1/3 of the initial value or when the 30 min sample showed the minimum concentration. On the other hand it was considered slightly abnormal when the 30 min concentration was > 90% of the initial value and the 60 min sample showed the minimum. The test was interpreted as severely abnormal when the minimum was not reached in 90 min.

Blood sugar was determined enzymatically by hexokinase and glucose-6-phosphate-dehydrogenase (17).

Serum insulin was determined by radioimmunoassay (8) free and antibody-bound insulin being separated by alcohol precipitation (The Novo Research Institute supplied human standard insulin hog 125 I-insulin and insulin antibody (serum from guinea pigs immunized by hog insulin) for this study). Triple determinations were carried out and the coefficient of variation was approx. 10% and approx. 6% at concentrations of 20 and 60 μ U/ml respectively.

RESULTS

The clinical data for 11 patients with varying degrees of renal failure are shown in Table I while Tables II and III give blood glucose and insulin concentration during glucose tolerance and tolbutamide tests. One patient (case 10) had by

Table II. Blood glucose and serum insulin concentration during oral glucose tolerance test in 11 patients with renal failure

Case no	Glucose in blood (mg/100 ml)				Serum insulin (μ U/ml)			
	(h)	0	1	3	(h)	0	1	2
1	89	240	174	5	15	180	132	8
2	84	181	103	51	10	99	60	26
3	104	241	148	77	8	54	40	29
4	96	225	115	84	12	142	38	16
5	113	201	121	104	31	250	88	56
6	104	168	176	113	23	36	78	51
7	87	227	207	153	11	93	136	102
8	87	119	90	74	7	118	38	36
9	81	214	168	76	27	137	80	36
10	107	185	117	102	16	162	144	126
11	79	164	148	108	11	111	91	56
Mean	94	197	138	91	14	171	84	51
s.d.	12	38	35	34	10	58	39	34

Table III Blood glucose and serum insulin concentration during intravenous tolbutamide test in 11 patients with renal failure

Case no	Glucose in blood (mg/100 ml)						Serum insulin (μ U/ml)							
	(min)	0	10	20	30	60	90	(min)	0	5	10	20	30	60
1	91	77	64	53	61	66	16	100	107	87	83	2	11	
2	87	74	66	48	61	70	7	51	57	43	32	15	8	
3	90	81	80	77	60	65	7	7	25	3	15	11	14	
4	91	89	76	67	47	55	8	75	89	93	81	23	20	
5	105	98	81	80	67	74	33	139	151	140	85	36	8	
6	100	91	91	85	65	69	16	53	56	43	9	1	10	
7	93	97	84	82	70	69	11	55	58	45	25	9	7	
8	83	76	50	30	40	54	18		166	160	86	24	16	
9	79	75	66	59	40	46	27		70	63	63	33	26	
10	98	97	80	69	46	52	15	86	83	90	70	30	18	
11	83	77	65	49	46	50	9		60	65	50	34	27	
Mean	91	81	74	64	55	61	15	73	84	78	56	23	17	
SD	8	9	13	17	11	10	8	35	43	42	27	10	8	

pertension. The glucose tolerance tests were normal in five cases (nos 1 2 4 5 and 10). The serum insulin value in the fasting patients averaged 14 ± 10 μ U/ml ranging from 0-31 μ U/ml. At the end of 1 hour a maximum of 36-250 μ U/ml was reached and at 3 hours the value ranged from 16 to 126 μ U/ml. As far as the tolbutamide tests were concerned six patients were found to have slightly abnormal responses (cases 3 4 5 6 9 and 10) and one a severely abnormal one (case 7). Serum insulin generally reached a maximum of between 25 and 166 μ U/ml 10 min after the injection of tolbutamide in order to fall gradually thereafter and return to the initial level in 90 min.

For comparison the findings in the nine non uraemic patients with normal glucose tolerance are as follows: clinical data listed in Table IV, blood glucose and insulin concentrations during

glucose tolerance and tolbutamide tests in Tables V and VI. The fasting insulin levels averaged 16-5 μ U/ml ranging from 10 to 25 μ U/ml, i.e. as in the uraemic subjects rising to 41-154 μ U/ml at 1 hour and thereafter returning to the initial values in another 2 hours.

In the tolbutamide test cases 1 2 4 5 and 8 reached their minimum glucose values in 30 min, i.e. the response was normal. In the other four it was slightly abnormal. In merely 5 min the insulin had reached the highest recorded value ranging from 37-80 μ U/ml then falling gradually and returning to the initial level in 90 min. As far as the course of the insulin concentration is concerned there was no difference between patients with normal and with abnormal tolbutamide response.

Tables VII-IX lastly illustrate the findings in 13 non uraemic patients with abnormal glucose

Table IV Clinical data for 9 non uraemic patients with normal oral glucose tolerance

Case no	Sex	Age	Diagnosis	BP (mm Hg)	Height (cm)	Weight (kg)
1		46	Duodenal ulcer	100/80	160	60
3	o	17	Colitis	110/70	178	72
4		47	Cardiac neurosis	150/70	161	53
5	o	22	Gastritis	100/90	166	60
6		60	Cholelithiasis	150/90	159	80
7		60	Hypothyroidism	110/80	149	54
8	o	41	Ectasis	100/80	176	76
9		53	Bronchitis	140/90	161	64
	c	71	Duodenal ulcer	145/0	151	8

Table V Blood glucose and serum insulin concentration during oral glucose tolerance test in 9 non uraemic patients with normal glucose tolerance

Case no	Glucose in blood (mg/100 ml)				Serum insulin (μ U/ml)			
	(h)				(h)			
	0	1	2	3	0	1	2	3
1	74	227	114	55	16	74	15	0
2	86	99	102	76	25	52	67	25
3	91	211	117	58	10	77	68	9
4	80	144	59	74	12	56	19	13
5	92	149	124	99	12	58	29	9
6	86	193	119	82	20	79	44	27
7	87	159	124	111	23	154	81	28
8	77	126	99	51	15	41	28	10
9	85	227	119	66	12	114	59	18
Mean	84	171	109	71	16	78	46	15
SD	6	46	21	16	5	35	24	10

tolerance but with a normal fasting blood sugar i.e. patients with latent diabetes. One (case 8) had hypertension. Fasting insulin ranged from 0–28 μ U/ml and during the glucose tolerance test reached a maximum of 38–198 μ U/ml in 2 hours at 3 hours it ranged from 2 to 157 μ U/ml. The tolbutamide tests were slightly abnormal in cases 1, 3, 4, 6, 7, 8, 9 and 13 and severely abnormal in case 2. In this group the serum insulin reached as an average a maximum of 23–142 μ U/ml 10 min after injection of tolbutamide and thereafter gradually returned to the initial levels in 90 min.

The course of the serum insulin level showed practically no difference between patients with normal and with abnormal tolbutamide tests.

The mean values for the three groups of patients are shown in Figs 1 and 2.

Table VI Blood glucose concentration and serum insulin during intravenous tolbutamide test in 9 non uraemic patients with normal oral glucose tolerance

Case no	Glucose in blood (mg/100 ml)						Serum insulin (μ U/ml)						
	(min)						(min)						
	0	10	20	30	60	90	0	5	10	20	30	60	90
1	78		66	51	53	66	10		45	51	40	20	11
2	83	79	70	64	68	68	19	69	58	35	38	11	76
3	88	84	72	63	47	66	11	52	44	36	30	18	12
4	79	70	58	51	63	66	14	37	36	35	35	26	6
5	92	89	73	58	70	71	13	80	61	41	31	16	17
6	111	86	80	67	54	68	13	78	85	83	53	30	3
7	81	111	72	62	61	71	11	70	64	65	46	78	17
8	73	69	49	39	56	62	7	48	44	29	8	11	5
9	87	111	75	69	59	67	16	66	64	48	34	22	15
Mean	83	80	68	58	59	67	13	63	56	47	35	11	19
SD	6	7	9	10	7	3	4	15	13	18	13	7	9

Table VII Clinical data for 13 non uraemic patients with abnormal oral glucose tolerance

Case no	Sex	Age	Diagnosis	BP (mm Hg)	Height (cm)	Weight (kg)
1	♂	29	Colitis	110/70	187	83
2	♀	66	Erythema nodosum	120/80	165	55
3	♀	41	Melaena	115/60	159	58
4	♀	53	Colitis	130/80	164	68
5	♀	32	Constipation	115/75	160	5
6	♀	22	Constipation	115/65	170	59
7	♂	59	Hodgkin's disease	110/80	160	0
8	♀	85	Cerebral arteriosclerosis	180/110	159	67
9	♀	67	Pulmonary malignancy	130/70	168	50
10	♂	54	Periarthrosis humero scapularis	175/110	170	80
11	♀	67	Colitis	195/100	16	69
12	♀	70	Cerebral arteriosclerosis	130/70	161	57
13	♀	68	Osteoarthritis	120/70	160	61

Table VIII Blood glucose and serum insulin concentration during oral glucose tolerance test in 13 non uraemic patients with abnormal glucose tolerance

Case no	Glucose in blood (mg/100 ml)				Serum insulin (μ U/ml)			
	(h) 0	1	2	3	(h) 0	1	2	3
1	88	216	150	61	18	48	45	11
2	88	159	131	64	9	11	39	9
3	86	176	153	63	9	178	151	34
4	97	244	181	64	0	108	78	25
5	100	182	148	86	4	34	38	2
6	90	179	144	80	13	56	56	28
7	96	204	146	81	21	52	43	21
8	92	216	234	226	19	80	150	157
9	8	171	150	102	12	78	100	42
10	78	2 0	210	120	28	165	198	126
11	90	2 0	182	104	18	111	115	32
12	88	231	207	91	19	129	94	26
13	77	182	132	43	4	49	40	6
Mean	88	200	167	92	13	83	88	41
S.D.	7	26	33	46	7	39	53	47

Fig 1 illustrates the findings during glucose tolerance. A comparison of the individual groups shows

1 Normal subjects versus non uraemic patients with abnormal glucose tolerance

The glucose values differed significantly at 1¹/₂ and 2¹/₂ hours ($P < 0.001$, $P < 0.001$ and $0.02 > P > 0.01$) while the fasting values and

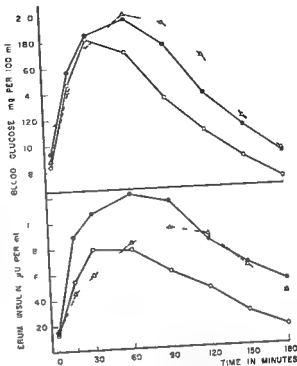


Fig 1 Oral glucose tolerance test: ● group A uraemics; ○ group B normals; ▲ group C patients with normal fasting blood sugar but abnormal glucose tolerance test. Abscissa: time in minutes. Ordinate: blood glucose mg/100 ml; serum insulin μ U/ml.

the 1 and 3 hour values did not differ. The insulin values also showed a difference at 2 hours.

Table IX Blood glucose and serum insulin concentration during intravenous tolbutamide test in 13 non uraemic patients with abnormal oral glucose tolerance

Case no	Glucose in blood (mg/100 ml)						Serum insulin (μ U/ml)						
	(min) 0	10	30	60	90		(min) 0	5	10	30	60	90	
1	80		69	61	61	69	13		26	14	15	9	11
2	94		79	75	62	59	2		35	36	29	15	13
3	88		79	69	56	71	12	94	99	11	47	11	9
4	85		75	66	52	62	10	31	27	7	11	7	
5	89		74	51	69	70	0	28	9	25	5	0	0
6	91	85	77	65	57	68	19	38	37	37	13	17	
7	93	96	74	55	63		0	53	41	40	7	19	0
8	99	96	83	73	61		2	19	3	19	17	11	4
9	87		77	70	58	67	18	55	5	4	34	11	17
10	83	72	58	47	53	61	27	153	14	91	56	31	8
11	9	81	70	56	57	65	1	70	75	37	17	11	
12		75	64	55	46	55	73	85	75	49	38	4	26
13	75	70	6	57	47	51	7	31	36	27	10	8	
Mean	85	83	72	64	57	64	13	51	31	4	8	15	13
S.D.	7	8	10	11	8	6	8	11	16	23	14	8	8

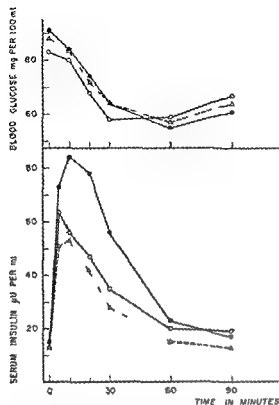


Fig 2 Intravenous tolbutamide test ● group A uraemics ○ group B normals △ group C patients with normal fasting blood sugar but abnormal glucose tolerance test Abscissa time in minutes Ordinate blood glucose mg/100 ml serum insulin μ U/ml

($0.05 > P > 0.02$) the patients showing the highest ulin values. The insulin curves are seen to run most parallel to the glucose curves.

2 Uraemic patients versus normal subjects

Fasting 1st, 2nd and 2^{1/2} hour glucose values were significantly higher in the uraemic than in the normal subjects ($0.05 > P > 0.02$, $0.01 > P > 0.001$, $0.05 > P > 0.02$ and $0.05 > P > 0.02$). Among the insulin values the 1st, 2nd and 3rd hour values were higher in the uraemic patients ($0.02 > P > 0.01$, $0.05 > P > 0.02$ and $0.01 > P > 0.001$).

3 Uraemic patients versus non uraemic patients with abnormal glucose tolerance

The 2 hour glucose values for the uraemic patients were significantly lower than for the other group ($0.05 > P > 0.02$). While the insulin values showed no significant difference at 2 hours the

30 min sample showed significantly higher levels in the uraemic patients ($0.02 > P > 0.01$).

Fig 2 presents the findings during tolbutamide tolerance. A comparison between the individual groups shows

1 Normal subjects versus non uraemic patients with abnormal glucose tolerance

Neither the glucose nor the insulin values showed any significant difference between the two groups. The only recorded difference was that the insulin level reached a maximum 5 min after the injection of tolbutamide in the normal subjects and not until 10 min after the injection in the other group.

2 Uraemic patients versus normal subjects

The glucose values in these two groups did not differ except for the fasting values which were significantly higher in the uraemic patients ($0.05 > P > 0.02$). The 20 and 30 min insulin values were higher in the uraemic patients ($0.05 > P > 0.02$) while the 10 min values showed no significant difference ($0.1 > P > 0.05$).

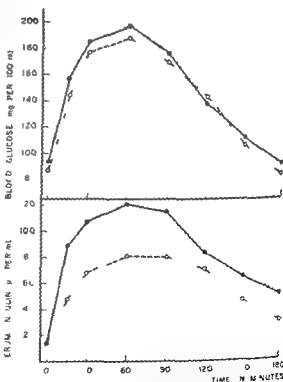


Fig 3 Oral glucose tolerance test ● uraemics ○ non uraemics Abscissa time in minutes Ordinate blood glucose mg/100 ml serum insulin μ U/ml

3 Uraemic patients versus non uraemic patients with abnormal glucose tolerance

While the glucose values in the two groups did not differ the 10, 20, 30 and 60-min insulin values were significantly higher in the uraemic patients ($0.05 > P > 0.02$, $0.02 > P > 0.01$, $0.01 > P > 0.001$ and $0.05 > P > 0.02$).

If all non uraemic subjects are considered together the mean curves are as shown in Figs 3 and 4. It is apparent from Fig 3 that the glucose levels during glucose tolerance tests are identical with the exception of the fasting values which are somewhat higher among the uraemic patients ($0.05 > P > 0.02$). Serum insulin was significantly higher in the uraemic patients at $1/4$, 1 and $1\frac{1}{2}$ hours ($0.02 > P > 0.01$, $0.01 > P > 0.001$ and $0.05 > P > 0.02$). From Fig 4 it is apparent that the glucose values during the tolbutamide test were also identical for both groups while the serum level of insulin was significantly higher in the uraemic patients at 10, 20 and 30 min ($0.05 > P > 0.02$, $0.01 > P > 0.001$ and $0.01 > P > 0.001$). The maximum was not reached until at 10 min while the non uraemic subjects reached their maximum in 5 min.

Lastly an analysis was made for correlation between the height of the serum creatinine and the increase in serum insulin during the tolbutamide test. No such correlation seemed to exist and the hypertensive uraemic patient did not exhibit a greater increase of insulin than the others but the increase persisted in spite of the decrease in blood glucose.

DISCUSSION AND CONCLUSION

Comparison of the glucose values found in the 11 uraemic and in the 22 non uraemic patients during oral glucose tolerance as well as during tolbutamide tests failed to show a significant difference. These findings are not in agreement with a number of previous studies (1, 6, 10, 11) but are confirmed by Westervelt and Schreiner (15) as well as by Tchobrousky et al (13) who demonstrated normal tolbutamide response in uraemic subjects. In a previous study of a large material Andersen and Friis (3) found no difference in glucose tolerance or tolbutamide tests between uraemic and non uraemic patients except in those who were under 60 years of age and had a serum creatinine exceeding 4 mg/100 ml.

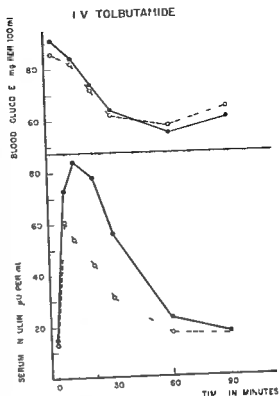


Fig 4 Intravenous tolbutamide test. ● uraemics ○ non uraemics. Abscissa: time in minutes. Ordinate: blood glucose mg/100 ml serum insulin μ U/ml.

The present series is too small for any similar conclusions.

Our finding that the insulin concentration which in the fasting state is as in normal subjects increases significantly more in uraemic than in non uraemic patients during oral glucose tolerance and tolbutamide tests is at variance with Tchobrousky et al (13) who found in uraemic patients a delayed insulin response to tests causing hyperglycaemia. Our findings also disagree with those of Briggs et al (4) who reported a delayed fall in the insulin concentration during oral glucose tolerance.

A comparison of the insulin concentration in the uraemic patients and in the 13 non uraemic patients with abnormal glucose tolerance shows that the insulin response of the uraemics was stronger during the tolbutamide test while during the glucose tolerance test only the 30 min values were higher. In the uraemic patients the maximum increase after oral glucose occurred at 60 min and during the tolbutamide test at 10 min the

corresponding figures for the 13 non uraemic patients were 90 and 10 min. The nine non uraemic patients with normal glucose tolerance reached on the average the insulin maximum in the glucose and tolbutamide tests at the end of 30 and 5 min respectively. The increase after oral glucose was less marked than in the 13 non uraemic patients with abnormal glucose tolerance.

The investigations indicate that the insulin response during the glucose tolerance and particularly during the tolbutamide test is increased in uraemic compared with non uraemic persons even though the latter have abnormal glucose tolerance. The explanation of this phenomenon may be that these patients have circulating substances which counteract the insulin or that their tissues are less sensitive to the action of insulin.

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IRON RESISTANT HYPOCHROMIC ANEMIA IN A SCANDINAVIAN FAMILY HETEROZYGOUS β -THALASSEMIA

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Abstract Four cases of heterozygous β thalassemia in three generations of a Norwegian family are reported. Hypochromic microcytic anemia with many target cells and a high serum iron concentration suggested the diagnosis hemoglobin starch-gel electrophoresis demonstrating an increased Hb A fraction and family studies confirmed it. It is concluded that iron resistant hypochromic anemia in individuals of apparently Scandinavian descent may be due to β thalassemia.

Thalassemia, a group of hereditary disorders of hemoglobin synthesis. There are two main types α and β thalassemia resulting from a defect in the production of either α or β chains of globin. In β -thalassemia the synthesis of adult hemoglobin (Hb A) is inhibited while the normally occurring Hb A and Hb F fractions may be increased. No abnormal hemoglobin is formed. The hypochromic and microcytic anemia of thalassemia results from a combination of defective hemoglobin synthesis and a shortened red cell survival.

This genetic abnormality occurs predominantly in the Mediterranean area, in the Far East and in Negroes. However, an increasing number of cases has been discovered in North European populations (1, 2, 3, 5, 7, 10). In 1958 Ytrehus (15) suspected the disease in two siblings of Norwegian-German parentage; a diagnosis was confirmed later by starch gel electrophoresis. The recent report of two Swedish families with the β thalassemia trait described the first cases among people of apparently Scandinavian descent (9). In this article we report four cases of heterozygous β thalassemia in a Norwegian family and discuss the problems of diagnosis.

METHODS

Routine hematological investigations were done by standard laboratory methods. Osmotic fragility of the red cells was investigated as described by Parpart et al. (8) slightly modified. (4). Hemoglobin solutions were prepared according to Dacie and Lewis (4) and adjusted to the same Hb level. Starch gel electrophoresis was carried out as described by Smithies (13) in Tris buffer (6) at pH 8.6. Hb A was reported normal or increased by visual comparison with control specimens. Fetal hemoglobin was determined by the method of Singer et al. (11).

RESULTS

Report of Cases

Fig. 1 shows that the thalassemia trait was found in all living generations of the family.

The proband (B1) was a 40-year-old man. A mild hypochromic anemia was first discovered when he was 20 years old. Two years later he was admitted to hospital because his anemia did not respond to iron therapy. At that time Hb was 12.4 g/100 ml and the serum iron was 170 g%. Physical examination was negative. In the following years he received numerous courses of oral and intramuscular iron without benefit. He was tired but regarded himself as healthy. He had never been icteric. Physical examination on the day of admission was negative except for areas with skin atrophy and diffuse pigmentation over both malleoli on the left leg and over the medial malleolus on the right leg. Varicosities were not present and he had probably never had thrombophlebitis.

The 80-year-old father of the proband (A1) was the oldest carrier of the trait. He originated from the South Eastern part of Sweden. No

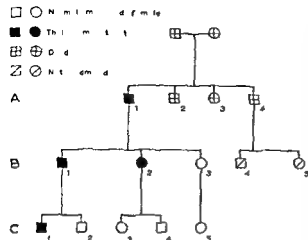


Fig 1 Pedigree of a Scandinavian family with the β thalassemia trait

foreign ancestors could be traced in the family for 150 years. He and his only affected grandchild a boy aged 15 (C 1) were totally asymptomatic and the physical examination was negative.

The fourth affected family member (B 2) was the 35 year old sister of the propositus, the mother of two healthy children. Like her brother she had been diagnosed as mildly anemic at the age of 20. In the following years Hb varied between 9.9 and 11.9 g/100 ml, the lowest value was recorded during her first pregnancy. She received iron orally and parenterally for eight years without effect until she discontinued the medication herself. At the time of our investigation she felt well and the physical examination was normal.

Hematological Findings

Table I shows that the two adult men were mildly anemic, the woman had a marginal Hb value while the boy's Hb was normal. How-

ever the RBC count was high in three of the four affected family members. The MCV and MCH were low in all patients but MCHC was normal. Examination of the peripheral blood film showed anisocytosis and poikilocytosis with pencil forms and other bizarre red cell shapes. The most striking finding, however, was the presence of target cells (Fig 2) especially in the anemic patients (Table I). Basophilic stippling of the red cells was occasionally observed. The osmotic fragility of the red cells was decreased in all patients (Fig 3). Serum bilirubin and haptoglobin were normal. The reticulocyte count was slightly elevated. Bone marrow aspiration in the propositus revealed normoblastic erythropoiesis and an increased amount of stainable iron. The sideroblasts were normal. Serum iron was clearly increased in the propositus, in the others it was in the upper normal range (Table I). Leukocytes and platelets were normal.

Fig 4 shows a representative example of the Hb electrophoresis which was performed in all family members. Although not quantitated in this study, the Hb A fraction in the four patients was obviously increased compared with healthy family members. The mean Hb F was 1.7% only the propositus had a level over 2%. No abnormal Hb components were found.

DISCUSSION

Two of the affected family members (A 1 and B 1) presented a triad which is virtually diagnostic of heterozygous thalassemia.

1. A hypochromic microcytic anemia with many target cells, occasional basophilic stippled red cells, pencil and other bizarre red cell shapes. Characteristically the morphologic changes in the red cells were far out of proportion to the degree of anemia.

Table I Hematological data of family members with the β thalassemia trait

Pedigree no	Sex	Age (y)	Hb (g/100 ml)	RBC ($\times 10^6/\text{mm}^3$)	MCV	MCH	MCHC	Retic (%)	Target cells (%)	Hb F (%)	Serum iron ($\mu\text{g}/100 \text{ ml}$)
A1	♂	80	13.3	6.5	63	0	32	2.2	15	1.7	127
B1	♂	40	12.7	5.2	77	24	32	1.8	25	2.5	195
B2	♀	35	11.7	5.5	65	23	33	2.4	12	1.1	1.8
C1	♂	15	14.1	6.6	88	21	33	1.8	2	1.5	115

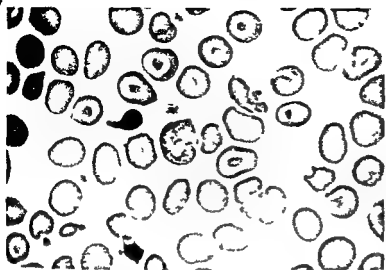


Fig 2 Peripheral blood film of the propositus (B1) showing anisopoikilocytosis, hypochromia, target cells and punctate basophilia. May Grunwald-Giemsa stain $\times 780$.

2 Normal or increased serum iron

3 Increased Hb A₁ fraction This is the most discriminative single observation and it is found in the majority of cases with the β thalassemia trait.

Hb F was slightly increased in the propositus (Table I) values below 2% are considered normal. However, less than 50% of cases have an increased Hb F fraction.

Family studies further confirmed the diagnosis. First, the hereditary trait of the disease was disclosed consistent with a dominant autosomal transmission. Secondly, the notable variability of this condition even within a small family group was revealed. B2 and C1 had all morphologic and biochemical characteristics of the trait, but were not anemic. This difference divides the

heterozygous state into two clinical forms: β thalassemia *minor* with slight to moderate anemia and few symptoms, and β thalassemia *minima* which is the asymptomatic trait without anemia.

The main differential diagnosis is iron de-

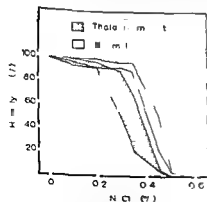


Fig 3 The decreased osmotic fragility of the red cells from family members with the β thalassemia trait.

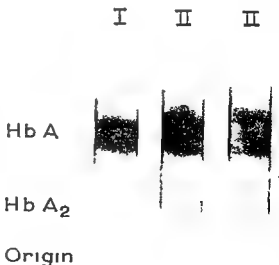


Fig 4 Starch gel electrophoresis of hemolysates from patients with the β thalassemia trait (II) and a normal family member (I).

Table II Hypochromic microcytic anemias

	Heridity	Target cells	Basophilic stippling	Osmotic fragility	Serum iron
Iron deficiency anemia	—	(+)	(+)	↓	↓
β thalassemia	+	++	+	↓	↓
Sideroblastic anemia	±	(+)	(+)	↓	↓
Lead intoxication	—	—	+++	↓	N
Pyridoxine responsive anemia	—	+	(+)	↓	↑

iciency anemia, the dominant cause of hypochromic microcytic anemia. Iron deficiency anemia is so common in Scandinavia that it would be clearly unrewarding to proceed with a full investigation of the possibility of thalassemia without the preliminary use of a screening test. In most cases the response to iron therapy is used to confirm the diagnosis. If treatment fails, however, it is important to make further investigations and consider other diagnostic possibilities (Table II). Unfortunately this is often not done, a fact well illustrated by the case stories of patients with β thalassemia. Two of the four patients reported here and 11 of the 21 patients in the Swedish investigation (9) had been treated with iron for several years without improvement. In iron deficiency anemia the serum iron is low and the Hb electrophoresis is normal. Target cells, cells showing punctate basophilia may be present but they are fewer than in thalassemia. A diagnosis of iron deficiency anemia refractory to oral iron therapy must be proved before it is accepted. According to Wintrobe (14) these cases are extremely rare.

Table II also presents three rare causes of hypochromic microcytic anemia. Among them the hereditary type of sideroblastic anemia may easily be confused with thalassemia ('pseudo thalassemia'). However, in this disease there is no increase in Hb A₂ and abnormal sideroblasts (ringed sideroblasts) are found in the bone marrow. Basophilic stippling of the red cells is the outstanding and characteristic feature in patients exposed to lead. Determination of the blood and urinary lead concentration and the coproporphyrin excretion confirm the diagnosis. Pyridoxine responsive anemia has many features in common with sideroblastic anemia. Diagnosis rests exclusively on a therapeutic trial.

This investigation confirms that β thalassemia

exists in apparently aboriginal Scandinavians. Unfortunately it cannot be decided whether this is due to a spontaneous mutation or to immigration. We can only maintain that foreign ancestors during the last 150 years could not be found and that our family was not related to the two recently reported Swedish families with the β thalassemia trait (12).

An improved diagnostic approach will reveal a number of new cases with thalassemia in Scandinavia in future years. The considerable contribution of manpower from Mediterranean countries will promote this development.

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Congress Announcement

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ACUTE EFFECT OF A SUSTAINED RELEASE PYRIDYL CARBINOL PREPARATION RONICOL RETARD® ON PLASMA FREE FATTY ACIDS

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Abstract The effect of two different single doses of a sustained release preparation of pyridyl carbinol Ronicol Retard® taken by mouth was studied in eleven patients. The results were compared to previously obtained data with identical experimental procedures for nicotinic acid and placebo.

The concentration of free nicotinic acid in plasma rose slowly after 0.45 and 1.05 g of Ronicol Retard® to reach a maximum level after 2 to 3 h and then declined to basal levels after 6 or more h.

The concentration of FFA in plasma was lowered to the same level as with nicotinic acid by both doses of Ronicol Retard®. In comparison to values obtained after placebo 0.45 g depressed the FFA levels for 1 / h 1.05 g for about 3 h.

When the FFA lowering effect vanished there was little rebound (overshoot) of FFA levels after Ronicol Retard® while nicotinic acid gave rise to a pronounced overshoot.

Flush similar to that seen after nicotinic acid occurred in all patients receiving 1.05 g of Ronicol Retard®. After 0.45 g not all patients showed flush and those who did had it to a slighter degree than after 1.05 g.

Compared to nicotinic acid pyridyl carbinol did not inhibit lipolysis in adipose tissue when added in vitro unless the concentration was 100 times greater than that of nicotinic acid. Even then the inhibition was much less pronounced than with nicotinic acid. This finding together with the appearance of free nicotinic acid in plasma after Ronicol Retard® suggests that this compound lowers plasma levels of FFA by being metabolized to nicotinic acid which then inhibits lipolysis.

The relationship of lowering of FFA the FFA overshoot and other possible effect of nicotinic acid to the lowering of the concentration of cholesterol in plasma is briefly discussed.

In 1955 Altschul and co-workers (1, 2) demonstrated that nicotinic acid in high doses lowers the concentration of cholesterol in blood plasma of animals and man. It is now evident that nicotinic

acid also lowers the other two major plasma lipid fractions contained in the plasma lipoproteins triglycerides and phospholipids (8, 12, 17). Nicotinic acid is now used with increasing frequency in the treatment of hyperlipoproteinemia.

In 1962 we demonstrated that nicotinic acid inhibits the mobilization of free fatty acids (FFA) from adipose tissue (11). It was postulated that this effect was one of several possible mechanisms by which this compound reduces the plasma lipoprotein concentration (4, 5, 14, 17, 18).

One of the disadvantages of nicotinic acid is that the high doses which are needed to normalize elevated plasma lipid levels frequently cause side effects usually from the gastrointestinal tract (1, 13). To reduce these side effects of nicotinic acid preparations with prolonged action like aluminium nicotinate have been tried (1). However it was not possible to abolish the side effects of nicotinic acid with these early preparations.

Several compounds chemically related to nicotinic acid have been shown to inhibit the mobilization of FFA from adipose tissue. Pyridyl carbinol is one of these and when given to man this compound lowers FFA levels (25). Ronicol Retard® a slow release preparation of pyridyl carbinol (24) has been reported to lower elevated plasma lipid levels in man (25, 29, 30). It appeared as if the dose of pyridyl carbinol needed to lower the plasma lipoprotein concentration was lower in this slow release preparation than that usually needed with plain nicotinic acid. With regard to the above mentioned effect of nicotinic acid and pyridyl carbinol on plasma FFA we considered it of in-

Table I Sex age height weight plasma lipids blood sugar intravenous glucose tolerance (k value) and plasma FFA in the 14 patients receiving Ronicol Retard^a

Case	Sex	Age (y)	Height (cm)	Weight (kg)	Cholesterol (mg/100 ml)	Phospho lipids (mg/100 ml)	Triglycerides (mmol/l)	Fasting blood sugar (mg/100 ml)	k value	FFA ^d (mmol/l) 0.45 g	1.05 g
A Ja	♀	72	157	47	284	330	0.76	83	1.39	0.39	—
G Z	♀	61	165	69	247	284	0.73	78	—	0.43	—
H J	♂	62	178	73	247	364	1.45	98	—	0.88	—
S G	♀	55	159	68	326	405	2.01	93	1.37	0.33	0.19
G S	♀	55	164	73	332	396	2.41	80	1.13	0.72	1.16
J J	♂	55	171	90	194	262	1.57	93	1.05	0.58	0.48
M A	♀	65	164	68	266	323	3.07	77	1.98	0.34	0.19
A J	♂	54	181	94	365	398	2.66	74	1.28	0.32	0.58
B S	♂	46	168	75	308	317	2.78	77	1.55	0.89	0.50
S B	♂	58	177	99	213	283	1.61	91	0.67	0.73	0.70
A K	♀	67	133	61	468	466	1.84	76	0.87	0.40	0.63
A Jo	♂	50	170	75	246	316	2.51	121	0.68	—	0.57
K K	♂	42	178	89	423	545	8.80	89	1.14	—	0.75
A A	♂	48	173	81	501	513	2.04	79	0.85	—	0.57
Range		41-72	155-178	47-99	194-501	262-545	0.73-8.80	74-121	0.68-1.39	0.32-0.89	0.19-1.16
Range previous study (17)		44-72	161-182	57.5-86.0	191-591	235-496	1.59-9.97	61-106	0.47-1.73	0.37-0.98 ^b	0.31-1.12 ^c

^a The FFA values were taken at the beginning of the test^b Range for placebo test^c Range for nicotinic acid test

terest to compare the acute effects of Ronicol Retard[®] and of nicotinic acid on plasma levels of FFA and nicotinic acid after a single dose of the two preparations. The technique used for this purpose was identical to one we developed previously to study the effects of nicotinic acid and pyridyl carbinol (17).

The effects of two doses of Ronicol Retard[®] were studied 0.45 g and 1.05 g. The first dose was given as this is the therapeutic single dose used in previous long term studies (30). The effect of the other dose 1.05 g was studied for comparison with 1 g of nicotinic acid which is the usual therapeutic single dose for nicotinic acid in treatment of hyperlipoproteinemia.

To evaluate whether pyridyl carbinol in itself inhibited the release of FFA from adipose tissue or whether it had first to be metabolized to nicotinic acid we also studied the effect of this compound on lipolysis in adipose tissue *in vitro*.

METHODS

The studies were done on hospitalized patients (Table I). Except H J and A J who had benign elevations of blood pressure with diastolic levels around 110 mm of Hg

(Table I) the only disease the patients had was ischemic cardiovascular disease either as a definite myocardial infarction (characteristic ECG and transaminase changes) at least six months before the study or as typical angina pectoris with ECG changes during exercise suggesting myocardial ischemia or intermittent claudication with oscillometric recordings typical of obliterating arterial disease. None had any complicating disease all were in good nutritional condition and the only symptoms present in some were ischemic pains on effort. No clinical diabetes (glycosuria, acidosis, vascular changes in eye grounds) was present, although one of them A Jo (Table I) had elevated fasting blood sugar (repeated determinations) but without increased plasma FFA concentration. Some of the patients had a lowered intravenous glucose tolerance in response to an intravenous glucose load. Most of them had moderate hyperlipoproteinemia (hypertriglyceridemia and/or hypercholesterolemia). All patients had been followed by us at the outpatient department for at least several months many for years. During that time they had had repeated blood samples withdrawn for lipid analysis which showed that the lipid levels were fairly stable. The lipid levels showed in no case at the time of the study any major deviation from the patient's usual level. No patient was on any special diet at the time of the study and in the hospital they had the ordinary hospital diet. Nor was any patient on drugs known to affect lipid metabolism or on antihypertensive drugs. They had not had nicotinic acid before. Table I shows that the patients given Ronicol Retard[®] were quite comparable to those previously given placebo and nicotinic acid under otherwise identical conditions (17).

Table II Plasma levels of FFA (mmol/l) before and changes after Ronicol Retard® nicotinic acid and placebo

Mean values \pm standard errors of the mean are given. The changes are calculated on the individual changes

Preparation	Time (hours) after administration of drug									
	Before	$\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3	4	5	6	7
Ronicol Retard® n = 11										
0.45 g	0.56 ± 0.09	-0.29 ± 0.08	-0.29 ± 0.06	* -0.28 ± 0.08	-0.14 ± 0.13	0.32 ± 0.16	0.55 ± 0.17	0.41 ± 0.09	0.39 ± 0.11	
1.05 g	0.52 ± 0.07	-0.18 ± 0.05	-0.29 ± 0.05	* -0.32 ± 0.05	-0.29 ± 0.05	-0.18 ± 0.11	0.12 ± 0.16	0.36 ± 0.16	0.59 ± 0.04	0.39 ± 0.03
Nicotinic acid n = 11 ^b										
1 g	0.53 ± 0.03	-0.22 ± 0.02	-0.31 ± 0.02	* -0.33 ± 0.03	* -0.31 ± 0.03	* -0.22 ± 0.04	* 0.76 ± 0.16	* 1.18 ± 0.14	1.00 ± 0.16	
Placebo n = 11 ^b										
1 g	0.59 ± 0.03	-0.15 ± 0.04	-0.17 ± 0.07	-0.10 ± 0.08	0.01 ± 0.07	0.12 ± 0.06	0.17 ± 0.07	0.19 ± 0.06	0.19* ± 0.07	

and n = 3 indicate statistical significance $P < 0.05$, < 0.01 and < 0.001 respectively for the changes from the basal level.
^b Data from (17)

The patients were carefully instructed about the purpose and possible side effects of the study. The experimental procedure was strictly standardized and has been described in detail earlier (17). In the morning after fast overnight a teflon catheter was inserted into an antecubital vein. A slow infusion of saline 0.9% kept the catheter patent and no heparin was injected. The patients who stayed in bed throughout the study were given a light breakfast, consisting of one cup of tea and two slices of bread without butter. The main purpose of the light breakfast was to reduce possible gastro-intestinal discomfort caused by the nicotinic acid preparations. About 45-60 min after the breakfast the first blood sample was withdrawn and the tablets were given by mouth. Blood samples about 10 ml each time were then drawn into heparinized syringes and in most instances without stasis. If stasis was necessary it was then used throughout the study. The blood was immediately processed for determinations of plasma FFA and nicotinic acid.

Fourteen patients received Ronicol Retard®. Six of them received either a dose of 0.45 g or 1.05 g and the remaining eight subjects received both doses on different occasions, 2 to 3 days apart. Plasma FFA were determined according to Dole (6) as modified by Trout et al. (77). The concentration of free nicotinic acid in plasma was determined as described previously from this laboratory (6). With this procedure pyridyl carbinol present in blood plasma gives about 10% of the optical density value of the same amount of nicotinic acid. In 21 samples from nine patients we separated nicotinic acid and pyridyl carbinol from each other by thin layer chromatography in a system with *n*-propanol/10% ammonium hydroxide (95/5) and subsequent elution and determination of these two compounds (6). The R_f value of nicotinic acid was 0.2 and of pyridyl carbinol 0.6 in this system. The sta-

tistical calculations were done as recommended by Sae decor (26).

For the *in vitro* experiments epididymal adipose tissue pieces were obtained from fed Sprague Dawley rats (180-200 g) and incubated *in vitro* as described in detail elsewhere (3, 5). The pieces were randomized between incubation flasks containing Krebs Ringer bicarbonate buffer 2% human serum albumin (Kabi, Stockholm, Sweden) and 0.1 glucose. Lipolysis was determined by enzymatic measurement of the amount of glycerol (28) released into the medium during incubation for 1 h at 37°C.

RESULTS

Plasma FFA

There were no significant differences in the mean plasma levels of FFA before administration of the two doses of Ronicol Retard® nicotinic acid or placebo (Table II). Already 30 min after administration of 0.45 g Ronicol Retard® the FFA had decreased from a mean concentration of 0.56 to 0.27 mmole/l (Figs 1 and 2, Table II). At about 1 $\frac{1}{2}$ to 2 h the FFA concentration started to increase (Table II) and reached a maximal level on the average 0.55 mmole/l above the initial concentration 4 h after the administration of Ronicol Retard®.

With 1.05 g of Ronicol Retard® the FFA concentration was depressed during 2-3 h (Figs 3 and 4, Table II). The FFA concentration then increased to a maximal level 0.59 mmole/l above

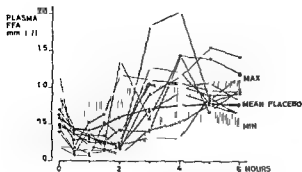


Fig 1 Comparison of individual values for plasma FFA levels after 0.45 g of Ronicol Retard[®] taken by mouth at 0 hours with mean value and range (max and min values) for values after 1 g of placebo by mouth (17)

the initial level at about 6 h after the administration of the drug

Nicotinic acid 1 g caused a significant depression of the plasma FFA concentration for about 3 h (Table II). There was then a pronounced overshoot up to maximal level of 1.71 mmol/l at about 5 h after the administration of nicotinic acid.

When placebo was given the concentration of FFA fell at 30 and 60 min (Table II) presumably at least partly as a result of the light breakfast. From one hour on the FFA level rose slowly and remained fairly stable during the last two hours.

The individual FFA values after 0.45 g of Ronicol Retard[®] are compared to the mean and range of values obtained after placebo in Fig 1 and after nicotinic acid in Fig 2. As seen up to about two hours nine of the eleven patients given 0.45 g were below the average placebo value. After 3 h most patients were above the mean

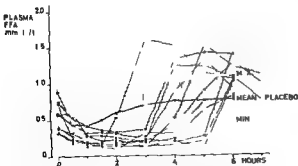


Fig 3 Comparison of individual values for plasma FFA levels after 1.05 g of Ronicol Retard[®] taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of placebo by mouth (17)

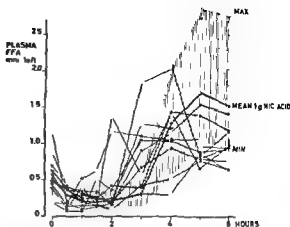


Fig 2 Comparison of individual values for plasma FFA levels after 0.45 g of Ronicol Retard[®] taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of nicotinic acid by mouth (17)

placebo level and four patients at 3 h and seven at 4 h were above the maximal FFA level seen after placebo. When compared to values found after 1 g of nicotinic acid (Fig 2) it is clear that the effect of 0.45 Ronicol Retard[®] on plasma FFA had a lesser duration in all but two patients. For instance at 2 and 3 h four and seven of the patients had FFA levels above maximal FFA values after nicotinic acid at corresponding times.

The individual FFA levels after 1.05 g of Ronicol Retard[®] are compared to mean and range for levels seen after placebo (Fig 3) and nicotinic acid (Fig 4). Fig 3 shows that in all patients up to 2 h, in ten up to 3 h and in seven up to 4 h

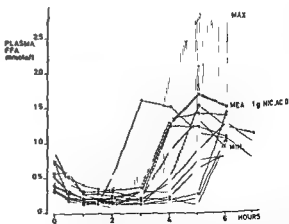


Fig 4 Comparison of individual values for plasma FFA levels after 1.05 g of Ronicol Retard[®] taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of nicotinic acid by mouth (17)

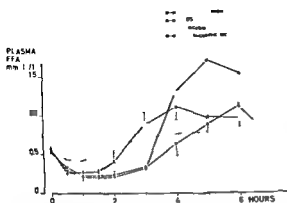


Fig 5 Comparison of mean levels of plasma FFA in patients after the two doses of Ronicol Retard[®] and of placebo and nicotinic acid. Bars indicate standard error of mean. \times — \times 0.45 g by mouth \bullet — \bullet 1.05 g \circ — \circ placebo \circ — \circ 1 g nicotinic acid

the concentration of FFA in plasma was lower than the mean level after placebo. At 4.5 and 6 h two five and four patients respectively showed an overshoot above placebo values. When the results are compared to the effect of 1 g of nicotinic acid Fig 4 demonstrates that with the exception of one case the FFA levels in the patients given Ronicol Retard[®] 1.05 g followed the nicotinic acid pattern up to three hours. Thereafter however they in general tended to be lower. It is especially noteworthy that the pronounced rebound effect seen after 1 g of nicotinic acid does not appear to the same extent after 1.05 g of Ronicol Retard[®] at least not within 6 to 7 h after administration. At 5 as well as 6 h all FFA values were below the mean value for 1 g of nicotinic acid.

The group mean levels of FFA after the two doses of Ronicol Retard[®] after nicotinic acid and after placebo are shown in Fig 5. The larger dose of Ronicol Retard[®] as compared to the lower gave the same FFA level up to about 2 h but at 3 and 4 h the levels were significantly lower. Fig 5 also clearly depicts the overshoot in FFA levels after 1 g of nicotinic acid and the almost complete absence of this phenomenon after 1.05 g of Ronicol Retard[®] at least up to 6–7 h.

Free nicotinic acid in blood plasma

The results of the 21 analyses of nicotinic acid and pyridyl carbinol by means of thin layer chromatography are given in Table III. It can be seen that in all instances early after administration

Table III Plasma levels of free nicotinic acid (NA) determined directly and after separation by thin layer chromatography (TLC) and plasma levels of pyridyl carbinol (PC) determined after separation by TLC

Plasma was taken at various times after 1.05 g of Ronicol Retard[®] by mouth

Case	Time (h)	NA Direct determination (μ g/ml)	NA Determined after TLC (μ g/ml)	PC Determined after TLC (μ g/ml)
K K	1½	54	55	26
K K	4	15	13	39
B S	1½	12	09	85
B S	3	39	25	37
A Jo	2	148	132	43
A J	1½	13	10	55
A J	3	48	43	77
J J	1½	34	32	55
J J	3	48	41	100
S G	1½	45	38	79
M K	1½	19	17	07
M K	1½	60	57	80
M K	3	47	48	23
M K	4	09	06	37
A K	1	04	0	—
A K	1½	24	20	23
A K	3	67	96	40
A K	5	13	17	17
A A	1½	25	19	0
A A	3	41	32	0
A A	5	04	05	0

(1½ h) as well as late (5 h) there is a good agreement with the direct analysis of nicotinic acid which includes the interference of pyridyl carbinol and the values obtained after separation of nicotinic acid from pyridyl carbinol on thin layer chromatography plates. The direct analyses give only slightly higher values even in the presence of considerable amounts of pyridyl carbinol. The explanation of this is that the interference of pyridyl carbinol is rather low as this compound only gives about one tenth as much color as nicotinic acid when analyzed in the same amounts.

With 0.45 g of Ronicol Retard[®] (Tables IV and VI) there was a slow increase in the plasma concentration of free nicotinic acid. The maximal mean level of 1.1 μ g/ml was reached after 2 h and the concentration was usually zero or almost zero after 6 h.

With 1.05 g of Ronicol Retard[®] (Tables V and VI) the maximal mean concentration of 5.2 μ g/ml was reached after 3 h.

After administration of 1 g of nicotinic acid on the other hand there was a rapid and marked

Table IV Plasma levels of FFA (mmol/l) and free nicotinic acid ($\mu\text{g/ml}$) determined directly after 0.45 g of Ronicol Retard[®] by mouth at 0 hours

Case	Time (h)	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	3	4	5	6
A Ja	Nic	0	0	0.3	1.0	0.5	0.1	0.1	0	0
	FFA	0.39	0.11	0.41	0.16	0.11	1.85	2.04	0.84	1.17
G Z	Nic	0	0	1.3	0.9	0.1	0.5	0.2	0	0
	FFA	0.45	0.14	0.15	0.14	1.38	0.58	1.44	1.40	1.19
H J	Nic	0	0.2	0.2	0.4	1.5	0.5	0.3	0.2	0.2
	FFA	0.88	0.41	0.35	0.34	0.16	0.91	1.15	1.55	1.42
S G	Nic	0	0.3	1.2	1.4	0.9	1.2	0.3	0.6	0.6
	FFA	0.19	0.18	0.10	0.13	0.19	0.68	0.94	0.80	0.76
G S	Nic	0	1.1	0.8	0.7	0.5	0.4	0.6	0.5	0.1
	FFA	1.16	0.19	0.52	0.63	1.17	1.11	1.05	1.07	0.83
J J	Nic	0.1	0.3	0.4	1.3	1.5	0.5	0.4	0.3	0.3
	FFA	0.48	0.37	0.34	0.23	0.25	1.10	1.07	0.97	0.95
M K	Nic	0	0	1.1	0.5	1.7	0.4	0	0	0
	FFA	0.19	0.08	0.07	0.52	0.37	1.05	1.04	0.90	0.92
A J	Nic	0	0	0.1	0.3	0.1	0.1	0.5	0.5	0.4
	FFA	0.58	0.29	0.29	0.76	0.22	0.32	0.30	0.87	0.64
B S	Nic	0	0.1	0.1	0.1	0.3	0.2	0.6	0.4	0
	FFA	0.50	0.37	0.25	0.25	0.43	0.40	1.43	0.67	0.98
S B	Nic	0.2	0	0	0.9	0.8	0.6	0.2	0.2	0.2
	FFA	0.70	0.42	0.32	0.26	0.19	1.28	1.21	0.81	0.94
A K	Nic	0.1	0	0.1	0.3	3.8	0.4	0.4	0	0.2
	FFA	0.63	0.39	0.20	0.15	0.09	0.43	0.52	0.77	0.63

increase in plasma concentration of nicotinic acid. The maximal level around 25–30 $\mu\text{g/ml}$ was seen already after 30–60 min. The nicotinic acid concentration then progressively decreased and reached μ levels at 6 h.

Relationship between concentrations of FFA and free nicotinic acid in blood plasma (Tables IV, V, VI and VII)

Already 30 min after 0.45 g of Ronicol Retard[®] the FFA concentration had decreased although

Table V Plasma levels of FFA (mmol/l) and free nicotinic acid ($\mu\text{g/ml}$) determined directly after 1.05 g of Ronicol Retard[®] by mouth at 0 hours

Case	Time (h)	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	3	4	5	6	7
S G	Nic	0	0	0.4	4.5	1.8	11.3	4.6	4.5	1.4	
	FFA	0.33	0.22	0.18	0.17	0.17	0.13	0.20	0.12	1.03	
G S	Ni	0.2	2.2	2.2	4.2	1.5	0.8	0.6	0.1	0.2	
	FFA	0.72	0.23	0.19	0.15	0.53	1.62	1.53	1.16	1.07	
J J	Nic	0.2	0.2	0.2	3.4	3.4	4.8	1.0	0.7	0.6	
	FFA	0.38	0.32	0.0	0.21	0.21	0.18	0.23	0.65	0.84	
M K	Nic	0.1	1.9	2.6	6.0	6.9	4.7	0.9	0.4	0.1	
	FFA	0.34	0.18	0.13	0.15	0.16	0.21	1.25	1.25	1.10	
A J	Nic	0.1	0.1	0	1.3	1.0	4.8	2.0	0.7	0.4	
	FFA	0.32	0.21	0.16	0.13	0.09	0.32	0.31	0.77	0.81	
B S	Nic	0	0.1	0	1.2	1.0	3.9	1.9	3.6	1.5	
	FFA	0.89	0.43	0.32	0.32	0.31	0.23	0.19	0.28	1.45	
S B	Nic	0	0	0.2	0.1	5.7	7.9	1.1	0.4	0	0.1
	FFA	0.73	0.54	0.28	0.20	0.22	0.13	0.51	1.55	1.24	1.13
A K	Nic	0.1	0.6	0.4	2.4	3.2	6.7	2.1	1.3	0.4	0.7
	FFA	0.40	0.28	0.18	0.13	0.14	0.13	0.15	0.21	1.09	0.76
A Jo	Nic	0	0	5.8	9.2	14.8	2.9	1.3	1.0	0.4	
	FFA	0.32	0.24	0.18	0.19	0.18	0.22	0.90	1.35	0.92	
K K	Nic	0.7	0.6	2.0	5.4	5.4	5.9	1.5	1.0	0.9	
	FFA	0.75	0.52	0.41	0.35	0.33	0.37	1.35	1.44	1.41	
A A	Nic	0	0	0.2	2.5	3.3	4.1	0.5	0.4	0.4	0.5
	FFA	0.57	0.60	0.28	0.28	0.25	0.20	0.44	0.92	1.30	1.00

Table VI Plasma levels of free nicotinic acid ($\mu\text{g/ml}$) after Ronicol Retard[®] and nicotinic acid by mouth at 0 hours

		0	$\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3	4	5	6
Ronicol Retard [®] $n = 11$										
0.45 g	Mean	0	0.2	0.5	0.7	1.1	0.4	0.3	0.3	0.2
	Range	0-0.2	0-1.1	0.1-1.3	0.1-1.4	0.1-3.8	0.1-1.2	0.1-0.6	0-0.6	0-0.6
Ronicol Retard [®] $n = 11$										
1.05 g	Mean	0.1	0.5	1.3	3.6	4.4	5.2	1.6	1.3	0.6
	Range	0-0.7	0-2	0-5.8	0.1-9.2	1.0-14.8	0.8-11.3	0.6-4.6	0.1-4.5	0.1-1.5
Nicotinic acid $n = 9$										
1 g	Mean	0.1	26.1	27.6	23.7	14.8	3.8	1.0	0.4	0.2
	Range	0-0.4	9.4-38.5	20.9-44.0	16.3-32.1	10.0-22.6	1.4-9.7	0.2-2.6	0-0.9	0-0.4

^a Data from (17)

there was still no measurable increase in free nicotinic acid concentration in the blood of several of the patients. This initial decrease may at least partly be explained by the light breakfast as there was a small initial fall in FFA also in the placebo group.

With 0.45 g of Ronicol Retard[®] the FFA levels remained depressed during 1-4 h and the nicotinic acid concentration varied between 0.1-3.8 $\mu\text{g/ml}$ during that time. The plasma FFA concentration started to increase when the concentration of nicotinic acid in plasma fell from a mean level of 1.3 to 0.5 $\mu\text{g/ml}$ (Table VII).

After the higher dose of Ronicol Retard[®] 1.05 g the plasma FFA levels were depressed during 1 $\frac{1}{2}$ to 6 h (mean 2-3 h) and the maximal nicotinic acid concentration in blood was seen after about 2-3 h. There were pronounced variations in the concentration of nicotinic acid in plasma between the different patients. Depressed FFA levels in the blood were seen with a nicotinic acid concentration varying from 0.1 to 14.8 $\mu\text{g/ml}$. Table VII shows that the FFA concentration started to rise when the nicotinic acid concentration fell from a mean level of 3.8 to 1.1 $\mu\text{g/ml}$.

After 1 g of nicotinic acid however the con

Table VII Concentration of FFA and free nicotinic in blood plasma when the plasma levels of FFA started to rise after the initial depression in FFA concentration after Ronicol Retard[®] and nicotinic acid

This rise was assumed to start when the FFA concentration increased 0.1 mmol/l or more from one hour to the next and the figures give the values at the start of the rise and the values one hour later.

		FFA mmol/l		Nicotinic acid $\mu\text{g/ml}$	
Time		Start of rise	One hour later	Start of rise	One hour later
Ronicol Retard® n = 11					
0.45 g	Mean	0.20	0.95	1.3	0.5
	Range	0.09-0.37	0.43-1.85	0.1-3.8	0.1-1.2
Ronicol Retard® n = 11					
0.45 g	Mean	0.22	0.91	3.8	1.1
	Range	0.12-0.37	0.44-1.45	1.0-7.9	1.0-7.9
Nicotinic acid n = 9					
1 g	Mean	0.46	0.96	2.8	2.7
	Range	0.10-0.32	0.37-1.93	1.4-26.0	0.1-13.0

^a Data from (17)

Table VIII Symptoms after administration of Ronicol Retard[®] 0.45 and 1.05 g by mouth at zero hours

Time (h)		0	$\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3	4	5	6	7
A Ja	0.45 g										
G Z	0.45 g			()							
H J	0.45 g			(^a)			C				
S G	0.45 g										
	1.05 g			(^c)	(^b)	(^a)	C				
G S	0.45 g		(^b)								
	1.05 g		(^c)		(^c)						
J I	0.45 g										
	1.05 g				()	(^c)		C			
M K	0.45 g	H	H	H(^a)	H	HC	H	H	H	H	H
	1.05 g		(^c)	(^c)	(^b)	()					
A J	0.45 g				()	(^b)					
	1.05 g				()						
B S	0.45 g				()	C	C	C	C	C	C
	1.05 g				(^b)	()	C	C	C	C	C
S B	0.45 g										
	1.05 g					(^b)	()				
A K	0.45 g					D(^c)					
	1.05 g				D(^c)	(^b)					
A Jo	—				(^c)	(^b)					
	1.05 g										
K K	—			(^b)	()				H	H	H
	1.05 g										
A A	—				(^b)	(^c)	()				
	1.05 g										

^a Slight flush in the face^b Marked flush in the face, neck and upper part of the body^c Marked flush in the face and the whole body

D = dizzy C = chilly H = headache

centration of FFA in plasma began to increase at higher plasma levels of nicotinic acid in general as shown in Table VII when the nicotinic acid concentration fell from a mean level of 8.8 to 2.7 $\mu\text{g/ml}$.

Symptoms (Table VIII)

Ronicol Retard[®] in a dose of 0.45 g produced a slight flush in 7 of 11 patients 30 min to 2 h after administration. With the higher dose 1.05 g a flush was seen in all subjects and it was more intensive and of longer duration.

With 1 g of nicotinic acid too there was always a marked flush for details see Carlson et al (17).

In vitro studies

The effect on lipolysis of the addition of nicotinic acid and of pyridyl carbinol to adipose tissue in vitro is shown in Table IX. Nicotinic acid at a concentration of 0.1 $\mu\text{g/ml}$ incubation medium

significantly depressed the release of glycerol by more than 50% from the incubated tissues as demonstrated earlier (4). Pyridyl carbinol however in concentrations of 0.1 and 1 μg per ml medium had no discernible effect on lipolysis. When the concentration was raised to 10 μg per ml medium pyridyl carbinol exerted a slight inhibition.

Table IX A typical result obtained when adipose tissue is incubated in vitro with nicotinic acid and pyridyl carbinol

Mean value (8 incubation flasks) \pm S.E.M. for the release of glycerol ($\mu\text{mol/g/h}$) is given

Addition to the medium	Nicotinic acid		Pyridyl carbinol		
	0	0.1 $\mu\text{g/ml}$	0.1 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$
	1.44 \pm 0.08	0.69 \pm 0.08	1.47 \pm 0.07	1.46 \pm 0.05	1.14 \pm 0.05

DISCUSSION

The data on the levels of free nicotinic acid in blood plasma after taking Ronicol Retard[®] or nicotinic acid showed quite different patterns. Plain nicotinic acid rapidly resulted in high peak levels soon levelling off. Ronicol Retard[®] on the other hand did not give early peaks and the blood concentration curve remained fairly flat. This difference might be ascribed to two factors. First the pharmaceutical preparation delays the resorption from the gastro-intestinal tract (24). Secondly pyridyl carbinol has to be metabolized in the body to be oxidized to nicotinic acid. This process however apparently occurs at a rapid rate in the liver (24). This suggests that the pharmaceutical preparation is the major factor responsible for the flat plasma concentration of the free nicotinic acid curve after Ronicol Retard[®].

Several studies including investigations of excess mobilization and inhibition (5, 7, 14) of FFA mobilization indicate that the flux of FFA from adipose tissue to the liver is an important factor in regulating the concentration of triglycerides in the liver as well as in plasma (10, 14, 15, 16). It has therefore been proposed that the inhibitory effect of nicotinic acid on FFA mobilization at least partly explains why nicotinic acid lowers the plasma lipoprotein concentration (4, 10, 11, 16).

It was reported that Ronicol Retard[®] in doses smaller than those usually needed with nicotinic acid effectively lowered the plasma cholesterol concentration in long term studies (30). If the cholesterol lowering effect of both nicotinic acid and Ronicol Retard[®] are related to lowering of FFA one might expect that Ronicol Retard[®] should induce a more long lasting decrease of the plasma FFA concentration than a similar dose of nicotinic acid. However from the present study it is evident that the duration of the lowering of FFA below placebo levels was almost the same after 1.05 g of Ronicol Retard[®] and after 1 g nicotinic acid but about one hour less with 0.45 g Ronicol Retard[®]. This latter dose is the dose which has been reported when given three times daily to lower cholesterol in a way similar to or even better than 1 g of nicotinic acid three times daily. If we accept that 0.45 g of Ronicol Retard[®] has at least a similar effect on plasma cholesterol levels to that of 1 g of nicotinic acid keeping in mind that no direct comparisons have been made

it seems difficult to reconcile the lowering effects of these two compounds on plasma FFA levels with their effect on the concentration of cholesterol in plasma. This suggests that effects other than inhibition of FFA mobilization may be related to the cholesterol lowering effect of nicotinic acid and/or pyridyl carbinol.

However nicotinic acid not only induces a fall in plasma FFA as the fall in FFA concentration after a single dose of nicotinic acid is always followed by a FFA rise above the initial level, an overshoot (11, 17, 23). It appears likely that during this overshoot period there is an increased FFA mobilization which might stimulate the hepatic production of lipoproteins thus counteracting the lipid lowering effect of nicotinic acid. As a matter of fact the overshoot of FFA levels occurring when the effect of nicotinic acid on lipolysis vanishes (11) has raised the question whether chronic treatment with nicotinic acid indeed lowers the turnover of FFA integrated over the 24 h period (17, 19, 21, 22). It may be quite relevant in the context of lowering of plasma cholesterol that we could demonstrate here that the FFA overshoot was less pronounced after Ronicol Retard[®] than after nicotinic acid. It is possible that this finding explains why Ronicol Retard[®] may be more effective than nicotinic acid in lowering the plasma lipoprotein concentration during long term treatment. Furthermore it should be stressed that one certainly cannot extrapolate from these studies performed under strictly standardized conditions and made as quiet and pleasant as possible for the patient to daily life situations.

The active substance in Ronicol Retard[®], pyridyl carbinol in contrast to nicotinic acid produced no inhibition of lipolysis in adipose tissue *in vitro* at concentrations of 0.1 and 1 µg/ml. The slight effect obtained at concentrations of 10 µg/ml could be explained if the preparation used contained less than one per cent of nicotinic acid as impurity. This lack of direct action on adipose tissues strongly suggests that Ronicol Retard[®] influences FFA mobilization after it has been metabolized to nicotinic acid. One can also conclude from the *in vitro* data that the levels of pyridyl carbinol in plasma very likely had no major influence on the FFA levels.

There were no correlations between the concentrations of FFA and nicotinic acid in the blood. This was most obvious at the time for the start of

the FFA overshoot. With 0.45 g Ronicol Retard* the mean nicotinic acid concentration decreased from 1.3 to 0.5 $\mu\text{g/ml}$ with 1.05 g Ronicol Retard* from 3.8 to 1.1 and with nicotinic acid from 8.8 to 2.7 $\mu\text{g/ml}$ when the mean FFA concentration increased in all three groups from around 0.2 to 0.9 mmole/l. In fact in one of the patients given nicotinic acid the FFA concentration increased although the concentration of free nicotinic acid in the blood still was at a level of 13 $\mu\text{g/ml}$. This indicates that the concentration of free nicotinic acid in the blood gives little or no information about the inhibitory effect of a nicotinic acid preparation on FFA mobilization. It is probable that determinations of nicotinic acid within adipose tissue would be more valuable in this respect. Also the difference in overshoot between nicotinic acid and pyridyl carbinol is noteworthy. Our results strongly suggest that it is not the inhibition of lipolysis per se that induces the overshoot. This result is compatible with the *in vitro* results obtained by Carlson and Micheli (9).

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DESTRUCTION OF ERYTHROCYTES DURING EXPERIMENTAL FEVER

Quantitative Aspects

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Abstract A simple analytical model has been worked out for quantitative assessment of temporary shifts in erythrocyte survival curves after labelling with radioactive tracers. This model was applied to ⁵¹Cr and DF³²P curves from rabbits exposed to experimental fever induced by either bacterial pyrogen, heated milk or external heating in a climatic chamber. Thereby a quantitative expression of the fever induced erythrocyte destruction was obtained.

In all three methods for elevating the body temperature experimentally the studies showed an increased loss of activity during the fever either in terms of the slope of the curve segment concerned with fever compared to the pre- or post-febrile periods or in terms of the deviation of the observed curves from the expected, spontaneous course in the rabbit. The maximum loss occurred soon after the initiation of fever and prolongation of the fever period did not cause a continued increase in the destruction of the labelled erythrocytes. A certain correlation was found between the initial action of heat and the magnitude of the erythrocyte destruction.

After the cessation of a short fasting period of fever the tracer curve again approached the expected spontaneous course explained by an age-dependent variation in the heat susceptibility of the erythrocytes. By using the age-distribution function for rabbit erythrocytes an explanation of the demonstrated differences in the simultaneous changes in radioactivity and haemoglobin or PCV is attempted.

It is concluded that elevated body temperature shortens the mean survival time of the red cells. The average reduction of the red cell mass in the present study was about 15% and the maximum loss about 35%.

Haemolysis is a contributory cause of anaemia in many diseases in which fever is a predominant clinical feature. In erythrocyte survival studies in rabbits using autologous erythrocytes labelled with Na ⁵¹CrO₄ or disopropylfluorophosphate (DF³²P) an increased destruction of red cells has been

demonstrated during experimental fever (5). These investigations are continued by the present study in which an attempt has been made to analyse the fever induced haemolysis quantitatively.

MATERIAL AND METHODS

Rabbits of both sexes weighing 2500-4000 g were used. Labelling of red cells was done by Na ⁵¹CrO₄ and DF³²P. The experimental methods used to induce fever were 1. i.v. injection of bacterial pyrogen, 2. i.m. injection of heated milk, and 3. external heating in a climatic chamber. For labelling procedure, sampling and radioactive measurements standard haematological studies and recording of temperature (Electro Universal Thermometer lent by the Control Laboratories of The Danish Pharmacist Association) compare the previous publication (5). The fever response was expressed as a Fever Index using the area below the temperature curve (½ Degree C × hours) and was determined by weighing the cut-out area.

Model in the quantitative analysis of fever induced erythrocyte destruction

The experimental results are illustrated in Fig. 1. The spontaneous course of the erythrocyte survival curve was observed during 10 days after Na ⁵¹CrO₄ labelling (0 days after DF³²P labelling). A period of fever was then introduced for 5-6 days with daily elevations of temperature lasting 10-14 hours (in induced hyperthermia only 6-8 hours). Fig. 1 demonstrates that there is a fall in the haemoglobin level and simultaneously a steeper drop of the survival curve (relative activity per ml blood) in connection with the fever. Quite rapidly reticulocytosis appears and after the fever has ceased the haemoglobin level returns to normal and the tracer curve again flattens out.

Normal erythrocyte survival in rabbits labelled with Na ⁵¹CrO₄ or DF³²P will appear curvilinear in a semi-logarithmic coordinate system with a slightly downward tendency. If this curve is divided into smaller time units, each of these units will to all practical purposes, present itself as a straight line. Accordingly the erythrocyte sur-

¹ Study performed during the tenure of a Research Fellowship from the Danish Anti-cancer League.

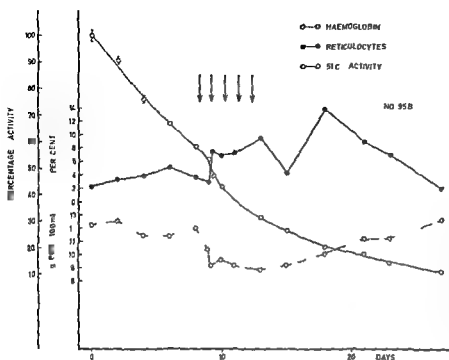


Fig 1 The influence of five days of pyrogen induced fever on the survival of ^{51}Cr labeled erythrocytes haemoglobin and reticulocyte values in a rabbit. Activity expressed as counts per ml blood in percentage of value at time zero

vival curves in the present material were divided into an initial pre febrile segment (I) a febrile segment (F) and one or two post febrile segments (P_1 and P_2). The equations for the relevant segments for each rabbit were determined by the method of least squares after log transformation of the value of radioactivity per ml blood. A similar division was made in the normal group and a comparison was performed by means of the slopes of the curve segments. To obtain a clearer expression of the induced loss of erythrocytes the initial curve segment which is the only expression of the spontaneous course in the fever exposed animals was extrapolated for all the rabbits through the subsequent time units. Comparison between the difference from this extrapolated line in the

normal rabbits and in the fever-exposed rabbits will give a description of the fever induced haemolysis. The percentage loss of activity as a result of fever was calculated for each rabbit at different times according to the equation

$$\frac{\left[F_{\text{fever}} \frac{C_0}{C_{\text{fever}}} \right] - F_{\text{normal}}}{F_{\text{fever}} \frac{C_0}{C_{\text{fever}}}} \cdot 100$$

(F_{fever} = calculated activity on extrapolated line from fever exposed animals F_{normal} = observed activity from fever-exposed animals C_0 and C_{fever} = corresponding mean values for the control group)

Table I Slope of curve segments in ^{51}Cr studies in rabbits before during and after experimental fever

Method of fever	No of animals	Periods						$\frac{a_P}{a_I}$	$\frac{a_P}{a_I}$
		a_I (day 1-10)	a_F (day 10-16)	a_P (day 16-25)	a_P (day 25-35)	$\frac{a_P}{a_I}$	$\frac{a_P}{a_I}$		
Controls	5	-0.0273 ± 0.0040	-0.0271 ± 0.0041	-0.0259 ± 0.0043	-0.0297 ± 0.0056	1.23 ± 0.07	1.17 ± 0.16	1.34 ± 0.21	1.25 ± 0.15
Pyrogen	16	-0.0703 ± 0.0036	-0.0397 ± 0.0161	-0.0208 ± 0.0072	-0.0749 ± 0.0070	1.94 ± 0.62	1.03 ± 0.28	1.25 ± 0.34	1.19 ± 0.21
Heated milk	5	-0.0700 ± 0.0026	-0.0438 ± 0.0090	-0.0706 ± 0.0077	-0.0736 ± 0.0044	2.21 ± 0.46	1.05 ± 0.43	1.19 ± 0.21	1.17 ± 0.21
External heating	4	-0.0158 ± 0.0053	-0.0324 ± 0.0119	-0.0205 ± 0.0045	-0.0201 ± 0.0082	2.25 ± 0.98	1.36 ± 0.5	1.37 ± 0.58	1.37 ± 0.58
Mean of fever groups		-0.0195 ± 0.0039	-0.0393 ± 0.0144	-0.0707 ± 0.0067	-0.0739 ± 0.0067	2.04 ± 0.65	1.08 ± 0.12	1.5 ± 0.36	1.5 ± 0.36
t test		$p > 0.10$	$p < 0.005$	$p > 0.10$	$p > 0.05$	$p < 0.001$	$p > 0.50$	$p > 0.50$	$p > 0.50$

Table II Observed ^{51}Cr activity at various times after experimental fever in rabbits in relation to hypothetical values obtained from extrapolation of pre febrile curve segment

Method of fever	No of animals	$t=16$ ($t_f=1$)	$t=21$ ($t_f=6$)	$t=25$ ($t_f=10$)	$t=30$ ($t_f=15$)
Controls	5	95.1 \pm 2.1	91.7 \pm 4.7	88.5 \pm 7.7	80.6 \pm 8.7
Pyrogen	16	82.4 \pm 10.7 ($p < 0.001$)	82.4 \pm 12.5 ($p < 0.025$)	83.1 \pm 16.4 ($p > 0.40$)	77.3 \pm 19.6 ($p > 0.70$)
Heated milk	5	78.1 \pm 7.2 ($p < 0.005$)	77.7 \pm 11.3 ($p < 0.05$)	78.4 \pm 16.9 ($p > 0.25$)	70.8 \pm 26.2 ($p > 0.40$)
External heating	4	81.3 \pm 11.2 ($p < 0.05$)	76.8 \pm 11.8 ($p < 0.05$)	74.3 \pm 12.0 ($p > 0.05$)	72.1 \pm 10.7 ($p > 0.20$)
Mean of fever groups		81.5 \pm 9.9	80.6 \pm 12.0	80.7 \pm 15.7	75.1 \pm 19.4
<i>t</i> -test		$p < 0.001$	$p < 0.005$	$p > 0.25$	$p > 0.50$

t =days after labelling t_f =days from end of fever period

RESULTS

I Studies by the ^{51}Cr Method

1 Slope of curve segments

The effect of a 5-day fever period upon the erythrocyte survival curve was studied on 25 rabbits starting 10 days after labelling with $\text{Na } ^{51}\text{CrO}_4$. In 16 rabbits the fever was induced by bacterial pyrogen in five by heated milk, and in four by external heating. The curves were fractionated into the following segments: I =1st-10th day F =10th-16th day P_1 =16th-25th day and P =25th-35th day. A normal group was divided in the same way. Table I gives the slopes for the relevant segments in the respective groups as well as the mean value for the fever-exposed groups. It is apparent that the fever period induces a significantly steeper course of the survival curve while the post febrile periods tend to show a smaller slope than the control group.

Even more distinct is that part of Table I which sets out the slopes for the fever and post fever periods in relation to the slope for the first period which represents the spontaneous destruction pattern for the individual rabbit. The relation between the 2nd and 1st period (a_f/a_1) is significantly higher in the fever groups while the corresponding value for the post febrile periods (a_p/a_1 and a_p/a_f) does not differ significantly from the normal material.

2 Differences of observations from an extrapolation of the pre febrile curve segment

Table II sets out the observed ^{51}Cr activities in the same material obtained by regression analysis of

the individual curve segments. The results are expressed in per cent of the hypothetical values from the extrapolated prolongation of the pre febrile segment calculated for the same time and starting immediately after the cessation of fever (16th day). From the results for the control group it is apparent that there is a tendency to a de-

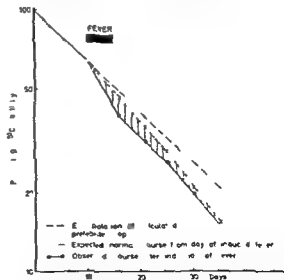


Fig. 5. Mean result from studies on the survival of ^{51}Cr labelled erythrocytes in 25 rabbits exposed to a five-day period of experimental fever produced by injection of bacterial pyrogen or heated milk or by external heating. The fever period starts ten days after the labelling. The figure demonstrates the basis for the calculation of lost activity by showing extrapolation of pre febrile curve segment the expected normal course (calculated from the survival of red cells in normal rabbits) and the observed course (cf text).

Table III *Loss of ^{51}Cr activity in haemoglobin and PCV (percentage) after five days experimental fever in rabbits*

Method of fever	Animal no	^{51}Cr	Haemoglobin	^{51}Cr	PCV	PCV- ^{51}Cr	PCV-haemoglobin
Pyrogen	1B	22.3	17.6	-4.7	22.2	-0.1	4.6
	2B	10.9	10.3	-0.6	11.8	0.9	1.5
	3B	15.5	17.1	1.6	19.4	3.9	2.3
	5B	3.3	18.3	15.0	27.2	18.9	3.9
	7B	47.0	34.8	-12.2	30.3	-16.7	-4.5
	8B	17.6	15.0	-2.6	16.7	-0.9	1.7
	9B	13.4	10.1	-3.3	6.2	-7.2	-3.9
	10B	0.3	4.4	4.1	8.5	8.2	4.1
	88A	16.5	19.7	3.2	20.6	4.1	0.9
	89A	0.6	8.1	7.5	11.4	10.8	3.3
	90A	6.1	15.1	9.0	18.2	12.1	3.1
	91A	9.1	8.1	-1.0	8.6	-0.5	0.5
	92A	5.6	13.7	8.1	17.5	11.9	3.8
	93A	8.8	13.0	4.2	14.3	5.5	1.3
	94A	16.4	31.7	15.3	41.7	25.3	10.0
	96A	17.1	19.7	2.6	21.6	4.5	1.9
Mean \pm s.d.		13.16	16.04	2.89	18.20	5.04	2.16
		± 11.09	± 8.06	± 7.25	± 8.89	± 9.91	± 3.34
Heated milk	14B	14.5	19.1	4.6	17.1	2.6	-2.0
	23B	13.2	17.5	4.3	16.7	3.5	-0.8
	24B	11.2	15.0	3.8	12.1	0.9	-2.9
	25B	20.5	22.5	2.0	21.2	0.7	-1.3
	26B	29.9	30.0	0.1	34.3	4.4	4.3
Mean \pm s.d.		17.86	20.82	2.96	20.28	2.42	-0.54
		± 7.55	± 5.74	± 1.89	± 8.48	± 1.61	± 2.16
External eating	12B	4.2	15.3	11.1	8.3	4.1	-7.0
	30B	29.3			30.0	0.7	
	42B	5.9	13.3	7.4	8.1	2.2	-5.2
	13B	15.3			14.3	-1.0	
Mean \pm s.d.		13.68	16.93	9.75	15.17	1.50	-6.1
		± 11.49	± 1.40	± 2.54	± 10.40	± 2.17	± 1.23
Mean of fever groups		14.14	16.93	3.46	18.13	3.95	0.85
		± 10.39	± 7.42	± 6.12	± 6.60	± 8.05	± 3.10
t test				$p < 0.02$		$p < 0.025$	
						$p > 0.40$	

crease with time corresponding to the shape of the normal survival curve. On the first day after cessation of fever the value of remaining activity is significantly lower in the fever exposed groups than in the control group. In other words the fever has entailed an increased loss of activity. The difference between the fever group and the control group decreases during the post febrile period when calculated for each day it is not significant after the 21st day. These results are illustrated in Fig. 2 which graphically shows the mean results for all rabbits of the fever group. It shows the pre febrile curve segment with the extrapolated prolongation also the expected spontaneous course calculated on the basis of the deviation from the extrapolated line in the control

group and lastly the observed course. Again there is a distinctly lower value of the observations after cessation of fever the difference between expected and observed course then decreases gradually but a difference remains throughout the experimental period. Table III lists the percental loss of ^{51}Cr caused by the fever calculated on the first day after its cessation. For the entire material this loss amounts to about 1.4% of the residual activity.

3 Changes in the survival curves during the fever and significance of the duration of fever

The changes in ^{51}Cr activity during the fever period itself were investigated by frequent samplings and by extending the fever period for 10 days. In order to avoid development of tolerance

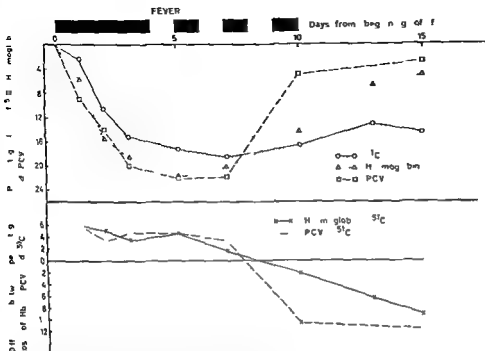


Fig 3 Upper curve Loss of ^{51}Cr activity and reduction in haemoglobin and PCV in relation to pre febrile values during and after a ten day period of pyrogen-induced fever (mean values from 3 rabbits)

Lower curve The difference in the relative changes in haemoglobin or PCV and ^{51}Cr activity during and after the fever

against the bacterial pyrogen afebrile days were interposed in the latter part of the fever period. The result is presented in Fig 3 which shows the mean values of fever induced ^{51}Cr loss in per cent at different times during and after the fever in three animals. The rate of loss is seen to be greatest initially and after approximately 4 days of fever there occurs only a minor additional loss. The total loss was in the same range as in the short lasting experiments.

4 Difference in the relative loss of ^{51}Cr and haemoglobin or packed red cell volume

Not only the percental loss of ^{51}Cr but also the percental fall in haemoglobin and packed cell volume (PCV) on the first day after the fever ceased for the three types of fever is seen in Table III. The difference between haemoglobin and ^{51}Cr loss and between PCV and ^{51}Cr loss varied somewhat but on the average it was significantly above 0. There was no difference between the alteration in haemoglobin and PCV. The findings are even more distinct when the difference

between the loss of haemoglobin and ^{51}Cr is followed during fever (cf Fig 3). The percental loss of haemoglobin exceeds the corresponding ^{51}Cr loss mainly at the start of the fever period but to a decreasing extent thereafter. Calculated on the basis of 12 connected sets of values for the first 5 days the difference is significant ($p < 0.001$). When regeneration of erythrocytes sets in as illustrated by the reticulocytosis in Fig 1 the values of haemoglobin and PCV return to normal and now the deviation of ^{51}Cr activity is greatest.

5 Relation between temperature response and erythrocyte loss

Table IV demonstrates the temperature response expressed as Fever Index on each of the 5 days of fever as well as the sum of heat action obtained by the three methods of inducing elevated body temperature. It is apparent that the later days of fever which contributed least to the loss of radioactivity and erythrocyte volume gave a rise in the temperature of the same order as the first days.

Fig 4 (right half) presents the relationship

Table IV Temperature response in experimental fever in rabbits with bacterial pyrogen heated milk and external heating

The response expressed as Fever Index (Δ degree C \times hours)

Method of fever	Dose/day (ml)	Animal no	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Pyrogen	0.25	1B	8.4	12.7	13.2	11.7	3.7	49.6
	0.50	2B	4.5	8.5	10.0	12.5	4.0	39.5
	0.50	3B	16.5	10.0	14.1	15.3	13.2	69.1
	0.75	5B	12.9	12.4	17.2	17.6	12.8	72.9
	0.75	7B	7.0	8.3	11.7	12.6	10.5	50.1
	1.00	8B	6.3	6.3	7.5	6.6	5.3	33.3
	1.00	9B	7.7	7.0	12.1	11.6	8.1	46.5
	1.00	10B	6.1	5.9	9.9	9.0	6.9	37.8
	1.00	88A	12.0	14.1	15.5	15.0	13.2	69.8
	1.00	89A	3.6	6.3	10.2	12.6	11.9	44.6
	1.00	90A	6.1	8.8	13.0	12.7	14.2	54.8
	1.75	91A	5.8	4.9	4.6	6.4	4.5	26
	1.75	92A	7.5	15.9	20.3	19.6	19.6	82.9
	1.75	93A	8.9	13.3	20.0	25.6	23.0	90.8
	2.50	94A	7.3	8.5	10.6	9.6	5.7	41.7
	2.50	96A	6.3	7.9	16.6	16.0	13.6	60.4
Mean			7.93	9.41	17.89	13.33	10.64	54.24
\pm S.D.			± 1.30	± 3.30	± 4.25	± 4.86	± 5.62	± 18.43
Heated milk	5.00	14B	7.9	10.0	12.9	14.1	18.4	63.3
	7.50	23B	10.7	13.1	10.6	25.2	8.3	68.1
	7.50	24B	10.0	9.6	13.7	21.7	20.0	75.0
	10.00	25B	8.3	7.2	15.2	12.5	8.9	52.1
	10.00	26B	11.2	11.9	12.5	22.8	18.9	77.3
Mean			9.62	10.35	12.98	19.27	14.93	67.16
\pm S.D.			± 1.45	± 7.26	± 1.68	± 5.57	± 5.77	± 19.68
External heating		12B	16.5	18.7	19.1	16.8	25.8	96.9
		13B	18.7	21.4	17.5	16.3	11.1	85.0
		30B	19.9	25.1	24.6	21.3	17.4	108.3
		42B	14.2	19.9	19.8	17.5	15.3	87.7
Mean			17.58	21.23	20.25	17.97	17.40	94.48
\pm S.D.			± 1.1	± 2.77	± 3.08	± 2.27	± 2.15	± 10.37

between temperature response and the percental loss of haemoglobin (logarithmic scale) after 5 days of fever induced by the three methods. There seems to be a certain correlation corresponding to the small heat actions, while above a Fever Index of about 60 a nearly constant loss of haemoglobin was seen. Analysis of the relationship between temperature response and loss of haemoglobin after 2 days of fever (Fig. 4 left half) shows that the results fall into two groups. In the experiments using bacterial pyrogen and heated milk there is a positive correlation between temperature response and the logarithm of the percental loss of haemoglobin, although not particularly good. When temperature was raised by external heating on the other hand a loss of haemoglobin was not demonstrated until at somewhat higher Fever Indices.

It must be emphasized that no correlation was found between the quantity of pyrogen administered and the loss of haemoglobin.

II Studies by the $DF^{51}P$ Method

The effect of pyrogen induced fever was studied on 6 rabbits after labelling of erythrocytes with $DF^{51}P$. The period of fever was started 20 days after the labelling and was continued for 6 days. Fig. 5 shows the percental reduction of $DF^{51}P$ and PCV in relation to fever, the loss of activity being determined on the same lines as in the ^{51}Cr experiments. The change in activity was at a maximum just after the cessation of fever and was, on the average, greater than in the ^{51}Cr studies (26.78% as compared with 14.14%, $p < 0.025$). The difference between observed and expected normal activity decreased in the same way as stated

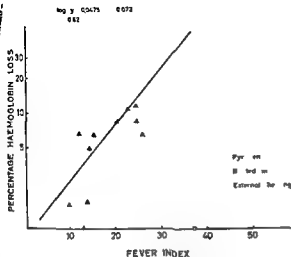
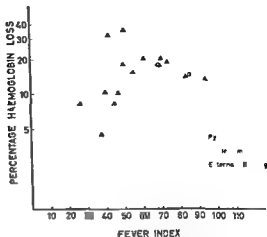


Fig. 4 Relation between fever response (Fever Index = degree C x hours) and relative reduction of haemoglobin (logarithmic scale) in fever induced by bacterial pyrogen, heated milk or external heating.

Left: Fever for two days there is a correlation between



Fever Index and the loss of haemoglobin in experiments with pyrogen and milk.

Right: After five days of fever the haemoglobin loss appears to reach a maximum.

previously after the fever period. In contrast to the ^{51}Cr studies the reduction in PCV although in the same range as in the ^{51}Cr studies ($p > 0.40$) was significantly less marked than the loss of activity during the entire period of fever ($p < 0.01$).

DISCUSSION

Alterations in the values of haemoglobin and PCV in connection with short lasting erythrocyte dam-

age must be considered a priori to be less suited for quantitative determination of the magnitude of destruction of red cells since as a matter of course these parameters must be the resultant of destruction and of possible compensatory regeneration. Instead it seemed likely that changes in erythrocyte survival curves after labelling with ^{51}Cr and DF^{32}P might form the basis for such calculation as it is assumed that activity released by erythrocyte breakdown cannot be reutilized.

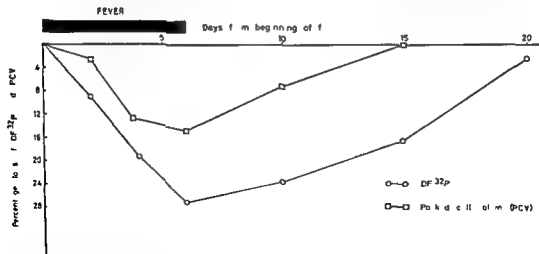


Fig. 5 Loss of DF^{32}P activity and the reduction in PCV in relation to pre-febrile values during and after a six-day period of pyrogen-induced fever (mean of 6 experiments).

The fever was maintained twenty days after the labelling of the erythrocytes.

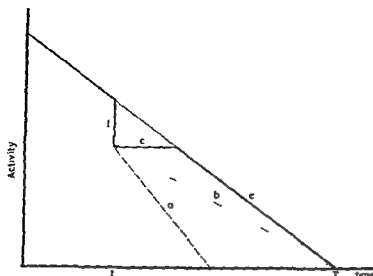


Fig 5 Theoretical course of erythrocyte survival after a transitory destruction of red cells (shown by line *l* at time *t*) depending upon the type of cell injury

Course *a* Injury to all red cells, some being destroyed at once and the remainder dying before normal life span (*T*)

Course *b* Acute random destruction with no injury to the surviving cells: the slope is less steep and the last labelled cell leaves the blood at time *T*

Course *c* Preferential destruction of the older cells with no injury to the surviving cells: in the first period after the destruction no labelled cell will leave the blood

The difficulty consisted in finding a basis for assessment which would render it possible to convert the demonstrated alterations in curves to an expression of the increased haemolysis.

Under normal conditions the erythrocyte breakdown in rabbits is due to a combination of senescence and random destruction. Since due to the latter type of destruction there is a skew age distribution function, the equation representing the loss of tracer labelling the entire erythrocyte population must be rather complicated (3, 4)

$$\frac{N_t}{N_0} = \frac{e^{-bt} - e^{-bT}}{1 - e^{-bT}} \quad (1)$$

(N_t = activity at time *t*; N_0 = activity at time 0; b = exponential coefficient for random destruction; T = maximum erythrocyte life span). Labelling with $\text{Na } ^{51}\text{CrO}_4$ introduces one more unknown owing to elution of Cr from intact erythrocytes

$$\frac{N_t}{N_0} = \frac{(1 - e^{-k(T-t)})e^{-(b+k)t}}{1 - e^{-bT}} \quad (2)$$

(k = exponential coefficient for elution of Cr)

However, these theoretical equations are applicable only to the pre febrile spontaneous segment of the present erythrocyte survival curves. Accordingly, a simplified model was introduced in analysing the experimental results, utilizing the fact that small segments of the survival curves may be interpreted approximately as a straight line in a semilogarithmic system. The fever-induced change in the curve is demonstrated partly by the steeper slope of the segment during the fever, partly by

the difference of the observations from a theoretical curve plotted by extrapolation of the pre febrile segment which represents the pattern of spontaneous loss of activity to the subsequent time units. A comparison between the different aberration of the observations in a control group and in the fever-exposed groups from this hypothetical curve afforded an expression of the percental loss in activity in relation to that expected during and after the fever.

By both tracer methods and by all three methods for experimentally inducing elevation of body temperature, the study showed an unmistakable fall in activity in the circulation during fever. This loss coincided with a reduction in haemoglobin concentration and PCV. The maximum loss occurred soon after the environmental change had been induced, and at continued elevation of temperature the loss of activity per time unit was in the same range as under normal conditions. When the fever ceased, the difference between the expected normal and the actually observed course of the erythrocyte survival curve decreased gradually. The explanation for this is presumably to be found by inspecting the theoretically possible courses of the survival curve after a transitory action which entails a certain loss of activity. In Fig 6 the normal survival curve is simplified as a straight line. At time *t* the animal is exposed to an abrupt loss of erythrocytes corresponding to segment 1. The action upon the erythrocytes is of a nature which affects all cells of the population irrespective of age in such a way that some cells die immediately and the re-

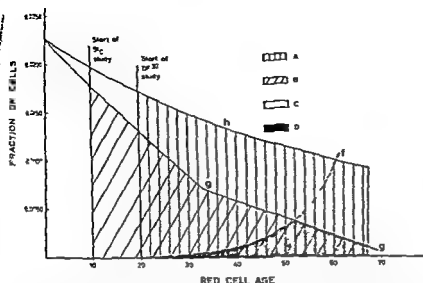


Fig. 7 Influence of a short period of fever on the erythrocyte population in rabbits illustrated on the basis of the distribution of red cells as a function of cell age (calculated from equation (3) $b=0.0133$ and $T=67$). Abscissa: different ages of red cells. Ordinate: fraction of cells at respective age. Total area: total erythrocyte population = total haemoglobin mass.

Area A Part of population labelled by $DF^{32}P$ at the beginning of fever period (twenty days after labelling). **f** Tentative line showing age-dependent difference in sensitivity to fever. This line cuts out area C which represents about 26% of area A and about 15% of total area (the

relative reductions in $DF^{32}P$ and haemoglobin respectively found in the study).

Area B Part of population labelled by ^{51}Cr at the beginning of fever period (ten days after labelling). This area is limited by a tentative line (*g*) representing difference in labelling intensity as a function of cell age (cf. text).

Area D The ^{51}Cr labelled fraction of cells lost during fever. Depending upon the course of line *g*, area B expressed in per cent of area B may be greater or smaller than the haemoglobin lost, represented by area C in per cent of total area under line *h*.

remainder die at varying time but earlier than expected, the last labelled erythrocyte will leave the blood before the time of normal life span (*T*) in accordance with course *a*. If the damage involves random destruction, i.e. does not alter the chance of the remaining cells of obtaining the usual life span, the curve after the cessation of the damaging action will present a flatter slope than normal and intersect the time axis at *T* (course *b*). If, however, the damage only involves old erythrocytes and the unaffected cells continue to have a normal life span, there will be no labelled cells destined to break down during the period immediately after the fever; thus the curve will be horizontal until it reaches the curve representing the normal course which it will accompany as far as *T* (course *c*). The course of the survival curves found in the present study after the fever period appear to fit in best with possibility *c*: the observed and expected normal course apparently coinciding before the normal life span of rabbit erythrocytes (about 60

days). This is in keeping with previous experiments (6) using two-generation labelling with two iron isotopes which clearly showed that the erythrocytes are not destroyed by fever until they are about 20 days old or more; that the destruction increases with advancing cell age and that the changes could not be explained by haemodilution. The quantitative difference between the experiments using ^{51}Cr and $DF^{32}P$ may then be explained by this rise in sensitivity with increasing cell age. The latter isotope at the time of fever is present in relatively older cells than the former and the reduction in activity will be greater in spite of practically identical change in PCV.

An attempt to explain the demonstrated differences in the relative reductions in radioactivity and haemoglobin or PCV is given in Fig. 7. This illustrates the total red cell population distributed in fractions with cells of different age. The equation for this function is derived from equation (1) by differentiation

$$a(t) = \frac{b e^{-bt}}{(1 - e^{-bT})} \quad (3)$$

$a(t)$ = fraction of cell mass with age = t

In this case the constants $b = 0.0135$ and $T = 67$ were used according to Brown and Eadie (1). As expected there is a decrease in number of cells with increasing age due to random destruction. The total area in the figure is an expression of the entire red cell population and equals 1. In Fig. 7 moreover a tentative line (f) is plotted for sensitivity to fever as a function of erythrocyte age starting at age 20 and showing the demonstrated increase in sensitivity with advancing age (6). In the experiments using $DF^{51}Cr$ the isotope is present in cells which are at least 20 days of age at the initiation of fever (area A). The experimental fever period has given a mean disappearance of 26 per cent of $DF^{51}Cr$ present and if a corresponding area (C) is cut out, using the line representing sensitivity due to cell age this will remove approximately 15% of the entire area under the curve which is measured as haemoglobin or PCV. These numbers are in accordance with the relative reductions in respectively $DF^{51}Cr$ and PCV found in the present study. The same reflections might be led to the experiments using ^{51}Cr . At the onset of fever the labelled cells are at least 10 days old and if the ^{51}Cr did represent the total cell mass with ages above this limit one would a priori expect a difference between the relative changes in radioactivity and haemoglobin or PCV in the same direction as in the $DF^{51}Cr$ experiments although less marked. The experimental findings however are exactly opposite as the mean value for relative loss of haemoglobin or PCV exceeded the ^{51}Cr loss especially during the initial part of the fever period. Studies have been made however which would indicate that the ^{51}Cr method does not label the erythrocyte population uniformly. Thus differential centrifugation with coarse separation of the erythrocytes into various age groups has shown that the youngest cells contain twice the ^{51}Cr of the average population and 3-4 times that of the content in the oldest cells (2). This difference of labelling intensity with various cell age has tentatively been illustrated by line g in Fig. 7 assuming that all the youngest cells are labelled, and depending upon the detailed shape of this function the fever induced loss of the ^{51}Cr labelled part of the erythrocyte population (area

D) expressed in per cent of total ^{51}Cr area (area B) may be sometimes greater and sometimes smaller than the removed haemoglobin (area C) amounts in per cent of the total haemoglobin mass, as was seen in the present study (Table III).

From the difference in the results obtained with ^{51}Cr and $DF^{51}Cr$ it is seen that the loss of activity is simple consequence of the age-dependent variation in sensitivity depends upon the placement of the fever period in relation to the time of labelling. As far as the ^{51}Cr method is concerned the loss depends as just mentioned also upon the presumed individual variation in the labelling intensity with different cell age. The measured changes in activity therefore served mainly to elucidate the erythrokinetics during fever while the best obtainable quantitative expression of the loss in the erythrocyte mass in the present study is the decrease in haemoglobin and PCV. The changes of these parameters will be underestimated if appreciable regeneration occurs during the fever.

Judged by the effect after a five-day period of fever there seems to be a maximum limit to the loss of haemoglobin (Fig. 4). This accords with the chronological course of the loss during the fever: it was shown that the greatest loss occurs at an early stage of the fever period and that a prolongation of the elevated temperature does not contribute much to the reduction. Considering the situation after a short time of action (2 days) there is a fairly good correlation between Fever Index and haemolysis in pyrogen and milk induced fever: the loss of haemoglobin appears to increase exponentially with increasing heat action, and there does not seem to be any threshold of susceptibility. The erythrocyte sensitivity is apparently less when elevated body temperature is induced by external heating. This may be due to a difference in size between core and rectal temperature during external heating and during the internal methods of fever.

CONCLUSION

On the basis of the present analysis the following conclusions may be drawn. Elevated body temperature during experimental fever is followed by a decrease in the mean survival time of the erythrocytes and according to this the total mass of erythrocytes will soon be to some extent reduced. In the rabbit this loss makes up about 15% of the

red cells. The further course depends upon whether the bone marrow under the new environmental conditions is able to compensate for this loss. If the bone marrow can produce erythrocytes only at the normal rate, the animal will remain anaemic to an extent corresponding to the primary loss of red cells. Otherwise erythropoiesis has to be increased in step with the reduction of mean survival time. In addition, the bone marrow must produce a quantity of red cells corresponding to the primary loss.

When using the previously selected constants for the breakdown equation, a loss of 15% of the red cell mass from the older part of the population corresponds to a reduction in mean cell survival time of from 44 to 37.5 days. Correspondingly, the daily percental influx from the bone marrow has to increase from 2.26 to 2.66, i.e. an increase of 18%. With the maximum erythrocyte loss of about 35%, which was demonstrated in the present material, the mean survival time will be 28.7 days, and the daily production required to maintain a normal haemoglobin level will be 3.48% of the erythrocyte mass, an increase of 54%. The increased demands on the bone marrow must be said to be modest compared with its normal capacity, but may be important in diseases involving relative inhibition of the bone marrow.

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DOUBLE BLIND TRIAL OF LONG TERM ANTICOAGULANT TREATMENT AFTER MYOCARDIAL INFARCTION

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Abstract A prospective double blind trial of long-term anticoagulant medication following myocardial infarction was carried out in Utrecht from May 1 1964 to January 15 1966. With the collaboration of all Utrecht cardiologists, all their patients with acute myocardial infarction were included in this trial after a 4-month interval to be distributed strictly at random over a phenprocoumon and a placebo group. Supplemental therapy and follow up were identical in both groups. Of the total of 138 patients included in the trial, 68 received phenprocoumon and 70 were given a placebo. One patient died in the phenprocoumon group and eight patients died in the placebo group. This difference is significant ($P < 0.01$). The difference in the number of reinfarctions was not significant (5 cases and 7 cases respectively).

After January 15 1966 when the difference between the two groups had become significant all patients were further treated with phenprocoumon. Nevertheless, the difference in mortality continued through 1966 with five deaths in the original placebo group and only one death in the original phenprocoumon group.

The mean thrombocyte level was 107×10^9 patients remained within the therapeutic limits ($5-15 \times 10^9$ thrombocytes) during an average of 91% of the entire period of investigation. The mean daily phenprocoumon dose was 3.1 mg. No serious haemorrhagic complications occurred.

The results of our study are consistent with those of several other recently published investigations. The intensity of the hypocoagulability attained is probably of decisive importance in this respect. However, correct calculation of the mean level of hypocoagulability is difficult.

The fact that in the original placebo group the results remained less favourable even after the switch to phenprocoumon emphasizes the necessity of long-term treatment with adequate doses of anticoagulants following myocardial infarction.

The value of long-term oral anticoagulant medication in coronary insufficiency has been a controversial and emotion-laden subject since the early years of this decade. In 1965 Merskey and Drapkin (18) concluded in an excellent review that the

data then available warranted no definite statement concerning the validity of this therapy.

In view of the risks and inconveniences entailed by long-term or even life-long anticoagulant medication we considered it very important to establish the validity of this therapy in more unequivocal terms. To achieve this goal we instituted a strict double-blind trial of long-term oral anticoagulant medication after myocardial infarction with the collaboration of all cardiologists practising in Utrecht and of the Utrecht Thrombosis Service.

MATERIAL AND METHODS

A. Choice of patients

The investigation started on May 1 1964 and was concluded on January 15 1966 at which time the material totalled 138 patients (124 men and 14 women) of whom 68 received anticoagulants while 70 were given a placebo. The total months of trial exposure was respectively 1317 and 1474 months.

Our study covered all patients in whom, since May 1 1964 a specialized cardiologist had definitely diagnosed either myocardial infarction or its recurrence on the basis of clinical, biochemical and ECG evidence. Once the diagnosis was established all were treated with acenocoumarol (Sintrom® Geigy) until their entry into the trial.

The patients were included in the trial procedure as soon as they were fully ambulant, i.e. after an average of four months. Data recorded for each patient included height and weight, age, sex, localization of the infarct and the occurrence of a drop in blood pressure and/or arrhythmia, the degree of peripheral vascular sclerosis, blood pressure, blood sugar and family history; the record also described the patient's condition at entry, fitness, cardiac decompensation, angina pectoris, ECG and therapy instituted.

All patients were given numbers in the order of their appearance. On this number determined whether a given patient was to receive the anti-coagulant or the placebo.

Table I Principal data on the composition of the phenprocoumon and the placebo group

	Number of patients	
	Phenprocoumon	Placebo
Age distribution y		
Men < 50	14	13
50-59	28	29
60-69	18	21
70	1	—
Women 50-59	3	3
60-69	4	4
Total	88	70
Average age	55.5	55.8
Total months of exposure	1317	1474
First infarction	63	62
Reinfarction	5	8
Severity of infarction		
Mild	34	30
Moderate	26	29
Severe	8	11
Localisation of infarct		
Anterior wall + spread (if any)	34	27
Other sites	34	43
Angina pectoris at entry		
Mild	17	17
Moderate	20	26
Severe	6	3
Cardiac decompensation	2	0
Peripheral vascular sclerosis		
Mild	17	17
Moderate	20	26
Severe	6	3
Diabetes mellitus	3	2
Overweight	16	18
Suggestive family history	9	9

For this purpose a random list had been made prior to the trial this list stipulated for each number whether anticoagulant or placebo was to be given. In this way we ensured an entirely random distribution of patients. The principal data on the comparability of the two groups of patients are presented in Table I. More detailed information can be supplied on the interested. The interval between the infarction and the entry into the trial is given in Table II.

B Anticoagulant medication

The anticoagulant used in the trial was phenprocoumon (Marcoumar[®] Hoffman La Roche Basle) the same manufacturer supplied the placebo-phenprocoumon tablets, which were identical to the phenprocoumon tablets in shape colour and taste. All tablets were dispensed by the nurses of the Thrombosis Service. Neither the patient himself nor the family doctor attending cardiologist or nurse were informed of the nature of the tablets the patient received. Only the project leader was aware of

Table II Comparability of the two groups as to interval between infarction and entry into the trial

	Interval in months							
	<3	-4	-5	-6	-7	-8	-9	Mean
Phenprocoumon	8	34	16	5	2	2	1	4.0
Placebo	2	27	20	15	5	1	0	4.5

this. The patients were given to understand that they participated in a study concerning tablets of a new type.

It was ensured that therapy group and placebo group were identical as in fluctuations in dosage and frequency of blood sampling for laboratory studies. Contrary to convention coagulation studies were not performed in the Thrombosis Service Laboratory lest the nature of the tablets become known and nurses be informed of the group to which the patient belonged. Determinations were instead made in the coagulation laboratory of the Utrecht University Medical Clinic. One of us (O J A T M) then informed the Thrombosis Service of the dosage to be employed and the date of reporting for the next follow up. The Thrombosis Service provided patients with the usual calendar showing the number of tablets to be taken each day and the new date of examination. Records were kept of the number of tablets dispensed. The number of tablets left to the patient at the end of each period indicated whether instructions as to dosage had been followed.

The phenprocoumon dosage was controlled with the aid of the thrombotest method (19). The goal was a dosage which confined hypocoagulability to between 5% and 15% thrombotest. For calculation of the mean dosage level we calculated the level of hypocoagulability for each patient for each day between all consecutive follow-ups, as a mean between the preceding and the subsequent thrombotest determination. On the basis of all these daily results the mean level of hypocoagulability was calculated over the entire period of investigation for the entire

Table III Incidence of unbalance of phenprocoumon dosage

No. of pts	No. of times unbalanced
11	0
17	1
23	2
6	3
7	4
2	5
0	6
1	7
1	8
68	134

Mean per patient 1.97 times

Table IV Localization of bleeding complications and thrombotest value during the bleeding episode

Localization	Thrombotest ()
Subconjunctival	5 ^a
Subconjunctival	9 ^a
Subconjunctival	6
Subconjunctival	5
Nasopharyngeal	14
Urogenital	3
Urogenital	7
Intestinal	10

Same patient

material. The mean level thus calculated was found to be 107^u thrombotest. Patients were regarded as unbalanced in terms of anticoagulant therapy when the thrombotest value was not between 5^u and 15^u. Throughout the period of investigation, hypocoagulability was unbalanced an average of 1.97 times per patient (Table III). Per patient, the thrombotest value was between 5^u and 15^u during an average of 91^u of the total period of investigation. The mean Marcoumar dosage per patient per day was 3.21 mg.

A total of eight haemorrhages occurred in seven patients (Table IV). This averages out at one haemorrhage per patient per thirteen years of treatment. These were all patients in the phenprocoumon group. An overdosage played a role in only one case. In no case was it necessary to interrupt phenprocoumon medication for a considerable length of time. In view of excessive diminution of the thrombotest value 15 patients were given vitamin K (Konaktion® Hoffman La Roche) in one patient this

was required on two separate occasions. None of these patients showed signs of a haemorrhagic diathesis. In three patients, administration of tablets was temporarily discontinued in view of indispensable dental therapy. Three patients were hospitalized for other reasons in the course of the trial. None of the abovementioned facts concern deceased patients.

C Cont and cations to participation

We excluded from the trial all individuals for whom one or several of the following contraindications were valid.

- 1 Age over 70
- 2 Diastolic pressure exceeding 10 mm Hg.
- 3 Severe hepatic and/or renal insufficiency
- 4 Disease associated with increased risk of haemorrhage (active peptic ulcer, haemorrhagic diathesis, etc.)
- 5 Concomitant malignant disease (carcinoma, etc.)
- 6 Valvular abnormalities and/or atrial fibrillation
- 7 Asocial behaviour and/or insufficient intelligence

D Control of patients

The attending cardiologist examined the patient at least every three months during the first year and subsequently at least every six months. Whenever a recurrence of myocardial infarction was suspected an ECG was recorded and one or several blood samples were obtained for determination of SGOT, SGPT and CPA activities. After each examination the cardiologist submitted to the project leader a follow-up form stating the patient's subjective and objective condition, the number of glyceryl trinitrate (Nitrobase) tablets used, ECG changes, results of biochemical determinations, if any, and a general evaluation of improvement or deterioration in the patient's condition.

In the case of a recurrence of myocardial infarction, the decision to start or withhold anticoagulant therapy was taken before the nature of the tablets used by the patient was disclosed. If anticoagulant therapy was indicated, this

Table V Review of deceased patients

	Placebo (P)	Phenprocoumon (M)	Age (y)	Sex	Infarction ranking	Clinical impression of infarction	Cholesterol (mg/100 ml)	Infarct localization	Angina pectoris at entry	Peripheral vascular atherosclerosis	Fitness at entry	Diabetes mellitus	Obesity	Interval between entry and death (days)
P	54	♂	1	Mild	214	Anterior wall	Absence	Severe	Undisturbed	No	Yes	352		
P	55	♂	1	Mild	272	Lateral wall	Moderate	Severe	Moderate	No	No	156		
P	59	♂	1	Mild	7	Inferior wall	Mild	Moderate	Undisturbed	No	No	182		
P	58	♂	2	Mild	280	Anterior & inferior wall	Moderate	Moderate	Moderate	No	No	160		
P	51	♂	1	Moderate	169	Anterior & lateral wall	Mild	Moderate	Moderate	No	No	102		
P	50	♂	1	Moderate	203	Lateral & posterior wall	Mild	Absent	Moderate	No	No	335		
P	45	♂	1	Severe	186	Inferior wall	Mild	Absent	Moderate	No	No	278		
P	61	♀	1	Mild	440	Lateral & posterior wall	Absent	Mild	Undisturbed	Yes	Yes	173		
M	58	♂	1	Mild	280	Anterior wall	Absent	Moderate	Undisturbed	Yes	No	98		

Table VI Comparison between deceased and living patients

	Total no of pats	Deceased	Living
Male	124	8	116
Female	14	1	13
Mean age, y	55.5	57.4	55.4
Hypertension			
Absent	116	8	108
Present	22	1	21
Overweight			
Absent	98	7	91
Present	40	2	38
Diabetes mellitus			
Absent	132	7	125
Present	6	2	4
Disability			
Absent	59	4	55
Moderate	74	5	69
Severe	5	0	5
Infarct localization			
Anterior wall with spread (if any)	61	4	57
Other sites	77	5	72

was given for at least three months whereupon the patient was returned to the therapy he had received prior to the recurrence

Evaluation of results

- criteria to be used in evaluating results were for
- 1) stated before the trial was started namely mortality
 - 2) number and severity of recurrences of infarction
 - 3) subjective and objective changes in the patients' condition as described on follow up forms (see above) by attending cardiologists ignorant of the nature of the tablets used by their patients

RESULTS

On January 15 1966 a total of nine patients had died eight of the deceased were in the placebo group and one was in the phenprocoumon group

The principal data on the deceased patients are presented in Table V Of the eight placebo patients six died a peracute death at home while the remaining two died a few hours after hospitalization for recurrence of myocardial infarction The patient from the phenprocoumon group died as a result of colic sepsis in prostatitis no fresh myocardial changes were found at the postmortem

The difference between the two groups is highly significant ($P < 0.001$) Comparing the deceased with the non-deceased in our small material we find no demonstrable influence of sex age hyper

Table VII Comparison of mortality with predicted mortality in The Netherlands population during the period May 1 1964 to Jan 15 1966

	Total no of pats	Deceased per Jan 15 1966	Predicted value	Tail probability in testing for over mortality
Phenprocoumon	68	1	1.7	0.45
Placebo	70	8	1.7	0.001

tension overweight and infarct localization of the six patients suffering from diabetes mellitus two died (Table VI)

Table VII compares the death risk in the group studied with that of a Netherlands population of comparable age distribution The comparison shows that the death rate in the placebo group is significantly higher than could be expected in view of the mortality in the Netherlands population This is not the case in the phenprocoumon group

The survival of the deceased patients after entry into the trial averaged 204 days (Table V) The shortest survival in the placebo group was 102 days and consequently there are no indications suggesting that abrupt discontinuation of Sintrom medication at entry into the trial has led to reactive hypercoagulability with increased thrombotic tendency

There was no significant difference in number of recurrences of myocardial infarction between the placebo group (7 patients) and the phenprocoumon group (5 patients)

Of the patients in the phenprocoumon group 52% felt improved and 13% felt deteriorated corresponding values in the placebo group were 42% and 14% In the cardiologists' judgement 30% of the phenprocoumon patients showed improvement and 12% showed deterioration corresponding figures in the placebo group were 21% and 15% (Tables VIII and IX)

Table VIII Subjective changes in the patients' condition (% of all follow up forms)

	Better	Unchanged	Worse
Phenprocoumon	52	15	13
Placebo	42	44	14

Table IX Objective changes in the patient's condition (■ of all follow up forms)

	Better	Unchanged	Worse
Phenprocoumon	30	49	12
Placebo	21	64	15

Table X Comparison of mortality with predicted mortality in The Netherlands population during the period Jan 15 1966 to Jan 1 1967

	Total no of pats	Deceased per Jan 1 1967	Predicted value	Tail probability in testing for over mortality
Phenprocoumon	65	1	0.9	0.59
Placebo	62	5	0.9	0.02

When the difference between the phenprocoumon and placebo groups had become significant on January 15 1966 the trial was discontinued and all patients were adjusted to phenprocoumon. Follow up system dosage and supplemental treatment remained unchanged. Although the original placebo patients were now treated with phenprocoumon the mortality in this group remained significantly higher than in the original phenprocoumon group (Table X). The difference in mortality was not only demonstrable during the first few months after the change to phenprocoumon: three of the five deaths of patients from the original placebo group occurred in November and December 1966.

DISCUSSION

Since Wright's publication in 1948 (28) on long term anticoagulant medication following myocardial infarction this therapy has been the subject of numerous studies. Several reviews have summarized the results of these investigations (12, 13, 17, 18, 23, 27).

In their review Merskey and Drapkin (18) in 1965 formed the conclusion that none of the papers thus far published had reported on studies which met requirements of optimal strictness. Such authors as Douglas (7) formulated the require-

ments to be met by such investigations as follows:

Double blind technique, random allocation of subjects into treated and control groups, determination of suitability for anticoagulant therapy prior to allocation into treated and control groups, identical therapeutic regimen in both groups except for the anticoagulant drug and objective criteria such as death rate for determining the value of the therapy.

Many investigators in The Netherlands and abroad (8, 9, 11) still doubt the value of long term or life long oral anticoagulant medication. Prior to our trial we too felt very doubtful concerning the validity of long term therapy. The majority of the participating cardiologists were accustomed to confining anticoagulant therapy to the first three months after myocardial infarction. Many patients in our phenprocoumon group therefore received anticoagulant therapy for a longer period than would have been the case had they not been included in the trial.

In view of the design and procedure of our trial (see Methods) we believe that we have met all the requirements which can be reasonably formulated for studies of this type. Our study was double blind. Our material can be considered representative for Utrecht and its environs because all Utrecht based cardiologists participated in the trial. The study covered all their patients with myocardial infarction in panel as well as private patients. The cardiologists diagnosed myocardial infarction and/or its recurrence on the basis of clinical, biochemical and ECG findings. Allocation to therapy of control group was strictly at random, simply on the basis of numbers which the patients were given in the order of their appearance. For each number it had been decided in advance whether phenprocoumon or a placebo would be given.

The contraindications to participation—e.g. severe hypertension, haemorrhagic diathesis etc.—cannot have influenced the distribution of patients over therapy and control groups for the patient was not allowed to enter the trial until he met preformulated criteria.

Supplemental therapy and follow up system were fully identical for the two groups. Prior to the trial mortality had been accepted as the principal criterion in evaluating results.

In patients admitted to the trial recurrence of myocardial infarction was diagnosed before the attending cardiologist knew whether the patient in

question was in the phenprocoumon or in the placebo group

Loeliger (14) demonstrated the great importance of adequate dosage in long term anticoagulant medication. In comparing the results of various studies therefore great importance must be attached to the mean level of hypocoagulability. However calculating this level is a precarious undertaking. A primary disadvantage is the presence of an asymmetrical distribution of thrombotest results above the desired level: the range is from 15% to 100% thrombotest while below this level the range is only from 5% to 1% thrombotest. This leads to a rise of the mean thrombotest level and consequently suggests a less favourable level of hypocoagulability.

If the mean dosage level is calculated not on the basis of thrombotest percentages but on the basis of clotting times determined and if the mean clotting time established is then expressed in thrombotest percentage then the asymmetrical distribution which likewise characterizes the clotting times leads to a low thrombotest percentage suggesting a very favourable level of hypocoagulability.

A second disadvantage lies in the fact that patients whose values are above or below the desired level of hypocoagulability are likely to be more often examined in follow up than patients who are firmly balanced at the desired level. Therefore the frequency of follow up on balanced patients and the frequency of follow up on unbalanced patients are factors which in part determine the mean level of hypocoagulability if this is calculated as the mean of all thrombotest results obtained or of a random sample of results. In order to avoid this second disadvantage we calculated—or each patient for each day between all successive follow ups—the level of hypocoagulability as the mean of the preceding and the subsequent thrombotest result (not the clotting time!). On the basis of all these day averages we calculated the mean level of hypocoagulability for the entire period of investigation and the entire material. The mean level of hypocoagulability thus calculated was 10.7% thrombotest.

So far as we know this method of calculation has not been used by other investigators even so the problem of an asymmetrical distribution of thrombotest results persists and the level of hypocoagulability attained is therefore perhaps best

compared on the basis of the mean dosage of anticoagulant used.

The mean phenprocoumon dosage per patient per day in our trial was 3.21 mg. Loeliger (14) found a mean daily dosage of 2.83 mg. This mode of comparison too has its disadvantages e.g. the dosage is dependent on dietary habits, climatological influences and the patients' average age. However the advantage that the mean dosage can be calculated in a simple and identical way by all concerned is not negligible.

Phenprocoumon was the anticoagulant used. In long term anticoagulant medication we prefer a long acting anticoagulant because our findings and results show like other investigations (3, 4, 22) that a much more stable balance can be achieved with a long acting anticoagulant. The thrombotest value per patient was not between 5% and 15% thrombotest on only 197 occasions; the patients were within the proper therapeutic limits for an average of 91% of the entire period of investigation.

Much sooner than we expected we were forced to discontinue the trial because the difference in mortality between therapy group and control group had become significant ($P < 0.01$) even in our relatively small series. One death in the phenprocoumon group compared with 8 deaths in the placebo group. The deceased included patients who were younger as well as patients who were older than 55.

Our small series warrants no definite conclusion as to the influence of sex, age, overweight, fitness at entry and infarct localization on the prognosis; however it is a conspicuous finding that two of the six patients with diabetes mellitus died.

The mortality in the phenprocoumon group does not differ from that in the Netherlands population of comparable age distribution. The difference in this respect between the placebo group and the Netherlands population is highly significant ($P < 0.001$).

The number of reinfarctions were relatively small in this respect; the difference between the phenprocoumon and the placebo group was not significant (reinfarction occurred in 5 and 7 cases, respectively).

The two groups did not significantly differ either in subjective or objective changes in the patients' condition. This was evaluated on the basis of quarterly reports submitted by the patients.

icipating cardiologists who described subjective and objective changes in the patient's condition without knowing to which group the patient in question belonged. Of the phenprocoumon patients 52% felt improved while 13% felt deteriorated. The corresponding figures in the placebo group were 42% and 14% respectively. In the cardiologists' opinion improvement was noticeable in 30% of phenprocoumon patients, and deterioration in 12%, the corresponding figures in the placebo group being 21% and 15% respectively.

Thus while neither the number of reinfarctions nor the subjective and objective changes in the patient's condition differed significantly in the two groups yet in these respects too the phenprocoumon group showed the best results.

The highly significant difference in mortality found in our trial between therapy group and control group within so short a time is surprising for the majority of studies using a comparable control group disclosed no difference in mortality. The British Medical Research Council (20, 21), Aspenstrom (1), Harvald (10), MacMillan (16) and Seaman (24), Bjerkelund (2) found a diminished mortality only in patients under 60 and Clausen (6) observed it only in patients under 55—and only during the first year following the infarction. The absence of a difference in mortality in a number of these studies is probably explained in part by less adequate hypocoagulability (14).

Our findings are consistent with those reported by Lovell (15) and Schnapper (26) who likewise observed diminished mortality in their therapy group. Loeliger (14) also found a difference in mortality in favour of his therapy group although in his myocardial infarction trial this was not significant. But if his data are combined with those of a simultaneously made double blind study of peripheral arteriosclerosis then the difference found by him is significant also. In this context it must be pointed out that patients were not included in his trial until at least a year after they had suffered an infarction.

After January 15 1966 all our placebo patients were switched to phenprocoumon about halfway through March 1966 all these patients had attained an adequate level of hypocoagulability. Follow up and supplemental therapy were continued in the same way. On January 1 1967 the number of deaths in the original phenprocoumon group had risen to two and that in the original

placebo group had increased to 13. This difference too is significant. This finding is the more surprising because the higher mortality in the original placebo group had led to selection to the disadvantage of the phenprocoumon group. The fact that, after return to anticoagulant medication the results in patients deprived of this therapy for a considerable period continue to be less favourable stresses the necessity of protracted and adequate anticoagulant therapy following myocardial infarction.

Our findings supply no answer to the question as to how long this medication should be continued but—also in view of recent data from the literature—we believe that very protracted or even life long medication is indicated.

In the placebo group the Sintrom which the patients had received in the acute phase of myocardial infarction was replaced by placebo tablets within a day. This is a very abrupt discontinuation of anticoagulant medication. We have found no indications suggesting that this abrupt discontinuation gave rise to reactive hypercoagulability with an increased thrombotic tendency (25).

Addendum: Since the preparation of this paper two years have elapsed. Follow up phenprocoumon therapy and supplemental treatment were continued in the same way. From January 1967 to January 1969 three patients died in the original phenprocoumon group in the original placebo group seven patients, making up a total mortality since the end of the trial of four and twelve patients respectively.

These results are very remarkable and intriguing and, as far as we know have never been reported before.

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EVALUATION OF FINE NEEDLE ASPIRATION BIOPSY SMEARS IN THE DIAGNOSIS OF LIVER IRON OVERLOAD

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Abstract Estimate of stainable iron in fine needle aspiration biopsy smears has been evaluated in relation to histological estimate of stainable iron in needle biopsy sections and in relation to liver iron in needle biopsy specimens analysed chemically. Histochemical iron was arbitrarily graded 0-4. The histochemical grading in sections agreed well with that in smears, but there was a high degree of overlapping between the chemical iron values in the different gradings. However it can be concluded that the smear method is valuable as a simple screening test in the diagnosis of liver iron overload: grade 4 in most cases indicates definitely increased liver iron concentration.

In the diagnosis of haemochromatosis indirect evidence of liver iron overload can be ascertained by several procedures such as uptake of radioactive iron in ferrokinetic studies or quantification of iron stores by chelating agents. For exact diagnosis however a study of liver biopsy specimen is necessary. The most reliable method is to analyse liver iron chemically which can be done on percutaneous needle biopsy specimens (4). Conventional needle biopsy technique (Mengham, Visslierman) however is not without risks and is therefore unsuitable as a screening test of liver iron overload.

By the use of fine needle aspiration biopsy technique as described by Soderstrom (5) liver puncture complications are virtually absent, and this type of biopsy can be used on wide indications without undue risks. Stainable iron in such fine needle biopsy smears has been demonstrated (1, 5) but no systematic evaluation of this method as a tool in the diagnosis of liver iron overload has been published. In the present study the estimate of stainable iron in fine needle aspiration biopsy smears was evaluated in relation to the estimate of

stainable iron in histological needle biopsy sections and in relation to liver iron in needle biopsy specimens analysed chemically.

MATERIAL

The study included 59 patients (53 men and 6 women). The biopsy indication was in most cases chronic alcoholism (33) or porphyria cutanea tarda (13). The rest of the patients were biopsied for suspected liver disease. Histologically steatosis was found in most, some had fibrosis of varying degree and some cirrhosis. In a few inflammatory changes dominated the histological picture.

METHODS AND PROCEDURE

After local anaesthesia the puncture was made in the mid axillary line in a suitable intercostal space (usually the 9th) during apnoea. The fine needle aspiration biopsies were performed with the instrument designed by Franzén et al (1). Needles with an outer diameter of 0.7 mm were used. The aspirate was immediately distributed in thin short smears on several slides and allowed to air-dry. The biopsy technique has been described in detail by Soderstrom (5). Directly after the fine needle biopsy aspiration biopsy with thicker needle (internal diameter 1.6 mm) was performed. A small part of this biopsy specimen was fixed in 10% neutral formaldehyde for 24 hours, embedded in paraffin and cut into 3 μ sections for histological examination. Most of the biopsy specimen was used for chemical analysis of non-haem iron.

The methods for staining haemosiderin in sections and for analysis of non-haem iron and alkali-soluble protein were the same as described earlier (4). The staining of haemosiderin in smears was done by the method described by Hansen and Weinfeld (3).

To assess the amount of histochemical iron in fine needle aspiration biopsy specimens, the smears were examined at 900 \times magnification under immersion (Fig. 1). Iron may be situated in parenchymal cells and Kupffer cells. The latter in contrast to the former are ar-

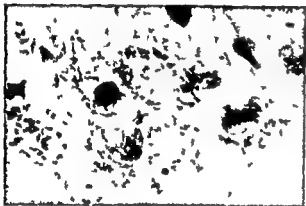
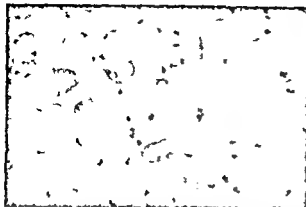


Fig 1 Fine needle aspiration biopsy smears showing iron granules in liver cells $\times 800$ (a) Grade 2 (b) Grade 3 (c) Grade 4

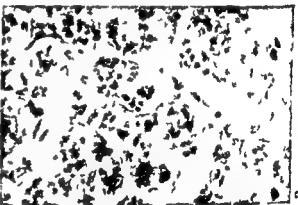
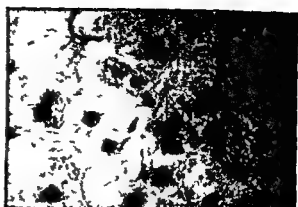


Fig 2 Corresponding findings in sections stained for iron $\times 800$ (a) Grade 2 (b) Grade 3 (c) Grade 4

very easily and by the smear technique phagocytized iron pigment is often spread extracellularly and is difficult to distinguish from artefacts. Therefore in this study only parenchymal cell iron was estimated. For fine needle aspiration smears the following grading was used:

Grade 0 No stainable iron or only isolated fine granules in all smears

Grade 1 Fine granular haemosiderin in a minority of the cell groups

Grade 2 Fine granular haemosiderin in a majority of the cell groups

Grade 3 Mainly fine granular haemosiderin but also large granules in most cell groups

Grade 4 Stainable iron very abundant in virtually all cell groups, to a large extent in the form of gross granules and aggregations of haemosiderin

The histological needle biopsy sections were examined at 600 \times magnification (Fig 2). The following grades were used (only parenchymal liver cell iron was graded)

Grade 0 No stainable iron or only isolated fine granules in the whole preparation

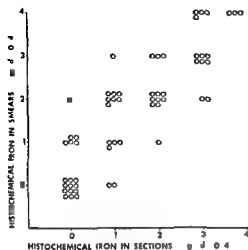


Fig 3 Grade of histochemical iron in fine needle aspiration biopsy smears as related to grade of histochemical iron in needle biopsy sections

Grade 1 Fine granular haemosiderin in single cells or in small scattered cell groups

Grade 2 Fine granular haemosiderin in a few or several cells present in most lobules

Grade 3 Mainly fine granular haemosiderin but also large granules present in the periphery of all lobules

Grade 4 Stainable iron very abundant in the major part of the lobule to a large extent in the form of gross granules and aggregations of haemosiderin

RESULTS

Relation of histochemical grade of iron in smears to histochemical grade of iron in sections (Fig 3)

The relation between the histochemical grade of iron in smears and in sections was studied in the whole series. As Fig 3 shows there was a good agreement between the two methods. In only two patients did the difference between the methods exceed one grade. Higher gradings in smears com-

pared with sections were more often encountered (21/59) than the reverse (5/59).

Relation between chemical iron (with protein as reference basis) and histochemical iron

The relation between chemical iron in thick needle biopsy specimens and histochemical iron in smears and in sections was studied in 55 patients.

The relation between chemical iron and histochemical iron in *fine needle biopsy smears* is shown in Table I and Fig 4. The mean non haemin iron concentration increased with higher histochemical grading but there was a wide range of iron concentrations in each histochemical grade. The mean iron concentration of grade II was not significantly lower than that of grade 1 ($0.10 > p > 0.05$) but significantly lower than that of grade 2 ($p < 0.005$) and significantly lower than the mean iron concentration of those with grade 1 and 2 ($p < 0.005$). There was no statistically significant difference between those with grade 1 and those with grade 2 but the difference between those with grade 2 and those with grade 3 was significant ($p < 0.005$).

The relation between chemical iron and histochemical iron in *histological needle biopsy sections* is shown in Table I and Fig 5. As in histochemical iron estimate of smears there was a high degree of overlapping of the non haemin iron values of the different histochemical gradings. The mean non haemin iron concentration of those with grade II however was significantly lower than that of those with grade 1 ($p < 0.05$). The difference between the mean iron concentration of those with grade 1 and those with grade 2 was not significant. The overlapping between the non haemin iron values of grade 2 and grade 3 was not so marked as in smears and there was no overlap between the

Table I Non haemin iron concentrations with protein as reference related to histochemical iron in smears and in sections

Grade of histochemical iron	Smears (mg/100 g protein) Mean \pm S.E. of mean			No	Sections (mg/100 g protein) Mean \pm S.E. of mean		
	No	Mean \pm S.E. of mean	Range		No	Mean \pm S.E. of mean	Range
0	14	103.4 \pm 11.8	34-168	17	110.2 \pm 11.1		34-213
1	10	154.2 \pm 24.9	90-328	13	167.4 \pm 17.2		74-263
2	16	170.1 \pm 15.8	74-296	12	182.9 \pm 10.5		82-328
3	9	264.3 \pm 27.7	169-411	10	361.3 \pm 39.0		209-580
4	7	1131 \pm 35.7	298-2845	3	2079		1446-2845

FOUR YEARS FOLLOW UP OF ASYMPTOMATIC ISOLATED PROTEINURIA DIAGNOSED IN A GENERAL HEALTH SURVEY

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Abstract The present work comprises a follow-up study on average four years after of 128 cases displaying isolated proteinuria of persistent (31 cases) intermittent (55 cases) or transient type (42 cases) in a general health survey. The check-up examination included a screening with questionnaire and analysis of morning urine sent in and also a clinical examination of cases displaying pathological urine findings or suspect case histories of urinary tract disease in the screening. In the clinical examination 89 individuals took part (0 cases with persistent, 33 with intermittent and 24 with transient proteinuria). Of these 89 cases only four (4.5%) had proteinuria in all three urine examinations which were performed. All these four cases had persistent proteinuria at the primary examination. Sixty-eight cases (76%) did not have proteinuria on any occasion during the follow-up six of whom had persistent proteinuria at the primary examination. Only 11 (12%) of the 89 clinically investigated displayed signs of urinary tract disease. Seven of these 11 cases with urinary tract disease belonged to the group with persistent proteinuria at the primary examination.

At health examinations it is not altogether uncommon to find proteinuria without other signs of urinary tract disease. In general greater significance is attached to an isolated proteinuria of this kind if it is constant than if it is intermittent (e.g. orthostatic) or transient. In recent years however it has been found at biopsies that even with the two latter forms of proteinuria pathological changes may be found in the kidney tissues.

Discovery of proteinuria often leads to costly extensive investigations with accompanying medical checks which may involve mental strain for the patient. It would therefore clearly be of great significance if one could prognostically evaluate an "isolated" proteinuria. With the object

of getting some idea of the prognosis for different forms of isolated proteinuria a follow-up study was therefore undertaken after three to five years. The subjects were individuals who at a general health examination were found to have proteinuria without other clinical signs of urinary tract disease.

MATERIAL AND METHODS

During 1962-1964 the Malmöhus County Council carried out a general health examination which from the nephrological viewpoint, primarily consisted of a screening for proteinuria with Albustix. Cases in which the results appeared to be positive were subjected to polyclinic investigation comprising a general medical examination, urine tests (Albustix, Heller's nitric acid ring test, Addis quantitative sediment, bacteria culture), ESR, Hb and in most cases determination of serum creatinine and intravenous urography. Kidney biopsy however was not performed—an investigation which would have been extremely valuable. In 1967 a follow-up was made of the 128 cases which apart from proteinuria had no other signs of urinary tract disease at the above mentioned health examination.

At the screening in 1962-64 only the Albustix test was performed, proteinuria being thereby defined as $\geq 2+$. On the other hand both Albustix and Heller's nitric acid ring test were used at the polyclinic investigation in 1962-64 and at the check-up in 1967. Proteinuria was at that time considered to exist with $\geq 2+$ Albustix plus a positive Heller's nitric acid ring test.

The 128 follow-up cases were divided into three groups from the results of the proteinuria tests which were carried out at the primary examination in 1962-64 in day urine in conjunction with the screening in the field, in day and night urine respectively at the subsequent examinations (Table I).

- 1 Transient proteinuria (proteinuria only at the time of screening) 42 cases, 23 women and 19 men. Mean age 26 years (range 16-60 years).

Table I Age distribution of the screened individuals

Age group	Transient proteinuria			Intermittent proteinuria			Persistent proteinuria		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
10-19	8	8	16	16	III	26	5	9	14
20-29	4	9	13	10	6	16	5	—	5
30-39	4	3	7	2	3	5	1	1	2
40-49	1	1	2	7	1	8	5	2	7
50-59	2	1	3	—	—	—	2	—	2
60-69	—	1	1	—	—	—	1	—	1
Total	19	23	42	35	II	55	19	12	31

Intermittent proteinuria (proteinuria at the screening and in day or night urine at the clinical examination) 45 cases 20 women and 35 men Mean age 24 years (range 15-46 years)

3 Persistent proteinuria (proteinuria at all three examinations) 31 cases 12 women and 19 men Mean age 27 years (range 16-60 years)

As can be seen from Table I the age distribution was similar in the different groups

The first part of the check up was done by post The patients answered a questionnaire about urinary tract diseases and at the same time they sent in a sample of urine morning urine to which was added four drops of 5% formalin (A) The following tests were made on the urine sent in Albustix, Heller's nitric acid ring test, talase test Clinistix, Haemastix, nitrite test and microscopic examination of sediment Individuals who exhibited pathological urine findings and/or had suspect case histories of urinary tract disease were called to a clinical examination which took place between 3-86 days later (on average 17 days)

At the clinical examination the same tests were made as previously partly on night urine which was tested for Addis quantitative sediment (B) and partly on day urine passed at the time of the examination after washing the outer genitalia with sodium chloride solution (C) A portion of the latter urine sample was cooled to +4°C and sent for semiquantitative culture to the central bacteriological laboratory of the hospital (Lead R Grubb MD) In 39 cases an X-ray examination was made comprising a survey of the urinary tract and intravenous

urography Thirty four of these cases had also been investigated at the primary examination in 1962-64 when a total of 121 out of 178 proteinuria cases had been X-rayed

Unfortunately at the follow up examination it was again not practically possible to perform kidney biopsies

RESULTS

Screening

A Analysis of morning urine sent in

1 Proteinuria As can be seen from Table II proteinuria as defined earlier was found in a total of 16 cases (12.5%) Thirteen (41.9%) of the 31 cases which in 1962-64 had what was designated persistent proteinuria had proteinuria in the urine specimen sent in 1967 Corresponding figures for cases with transient proteinuria were three (7.1%) out of 42 while none of the 55 cases with intermittent proteinuria displayed proteinuria in this urine sample

2 Pyuria (>5 WBC/HPF) occurred in a total of five cases (3.9%) The frequency was the same in the different proteinuria groups

3 Haematuria defined as >2+ Haemastix was found in altogether three cases (2.3%) one case in each proteinuria group

Table II Results of the 1967 screening examination

Group of proteinuria	No examined	Proteinuria		Pyuria	Haematuria	
		No	%	No	No	%
Transient	42	3	7.1	47	1	2.4
Intermittent	55	—	—	2	1	1.8
Persistent	31	13	41.9	1	1	3
Total	128	16	12.5	5	3	2.3

Table V Results of analysis of freshly voided urine at the clinical examination

Group of proteinuria	No examined	Proteinuria		> 10 ⁵ bact/ml urine	
		No		No	%
Transient	24	3	12.5	—	—
Intermittent	43	3	6.7	2	4.4
Persistent	20	6	30.0	2	10.0
Total	89	12	13.5	4	4.5

D Blood analyses

1 Serum creatinine Two of the cases with persistent proteinuria had increased serum creatinine values (1.3 and 1.7 mg% respectively). All cases with intermittent or transient proteinuria displayed normal values.

2 ESR Of the 89 examined 15 (16.9%) had an ESR in excess of 15 mm/1 h and three more than 30 mm/1 h.

3 Antistreptolysin titre Thirty seven (41.6%) of those examined had an antistreptolysin titre of more than 140 IU (the normal limit at this bacteriological laboratory) and 12 cases (13.5%) more than 500 IU. These latter cases were distributed equally among the proteinuria groups which gave a frequency of 20% for persistent proteinuria, 9% for intermittent and 17% for transient proteinuria (See Table VI).

E Other investigations

1 Blood pressure Two cases of hypertension were recorded in the group with persistent proteinuria but no significant difference was found between the different groups for either diastolic or systolic pressure.

2 X-ray examination At the health survey in 1962-1964 X-ray examinations (urinary tract survey and intravenous urography) were made in 121 of the 128 cases which initially took part in

the follow up study in 1967 with normal findings as the result. In 1967 39 cases were X-rayed 34 of which had also been investigated in 1962-64. No pathological findings were recorded at the 1967 examination either.

F Persistent, intermittent and transient proteinuria at the 1967 examination

If the cases which took part in the clinical examination in 1967 were grouped according to the number of occasions they displayed proteinuria (transient proteinuria 1 occasion, intermittent 2 occasions, persistent 3 occasions) it was found that only four cases (4.5%) of the 89 examined had persistent proteinuria, three (3.4%) intermittent and 14 (15.7%) transient proteinuria. The remaining 68 cases (76.4%) did not have proteinuria (according to our definition) at any time. The four cases which had persistent proteinuria at the follow up examination belonged to the 20 cases which in 1962-64 were classified as persistent proteinuria. Of the remaining 16 cases in this group six did not have proteinuria at any one of the three examinations in 1967 while eight had transient and two had intermittent proteinuria. Thus only one fifth of the cases which at the first examination displayed proteinuria at all the examinations still had constant proteinuria 3-5 years later and of the 69 cases

Table VI Results of blood analysis at the clinical examination

Group of proteinuria	No examined	ESR > 15 mm/1 h		Creatinine > 1.2 mg%		Antistreptolysin titre > 140 IU	
		No	%	No	%	No	%
Transient	24	4	16.7	—	—	10	41.7
Intermittent	43	6	13.3	—	—	18	40.0
Persistent	20	5	25.0	—	10.0	9	45.0
Total	89	15	16.9	—	4.5	37	41.6

Table VII Number of individuals with no proteinuria and with proteinuria on one two or three occasions at the clinical examination

Tests in 1967	Frequency of the different forms of proteinuria 196-1964			
	Transient proteinuria	Intermittent proteinuria	Persistent proteinuria	Total
No proteinuria	21	41	6	68
Proteinuria once	3	3	8	14
Proteinuria twice	—	1	2	3
Proteinuria thrice	—	—	4	4
Total	24	45	20	89

which in 1962-64 displayed transient or intermittent proteinuria none had become persistent at the follow up study. Sixty two of these cases (89.9%) did not have proteinuria at any time during the follow up.

G Clinical assessment

Seventy seven (87%) of the 89 cases subjected to closer examination were considered to show no signs of urinary tract disease. When taking into account the initial distribution into the different proteinuria groups it was found that 65% of the persistent proteinuria group, 93% of the intermittent group and 92% of the transient proteinuria group were considered to be healthy. In the persistent proteinuria group were noted two certain and one suspected case of urinary tract infection, one case of chronic pyelonephritis, two cases of hypertension and one additional case who had had nephrolithiasis between the examinations.

Two cases of urinary tract infection and one case with a history of repeated attacks of cystopyelitis were recorded in the intermittent proteinuria group.

Among the cases with transient proteinuria there was one with isolated haematuria and one case of gonorrhoea. This latter case would not appear to be of interest in this connection.

The four cases displaying persistent proteinuria both in 1962-64 and in 1967 were assessed as being clinically healthy apart from the proteinuria and they were not subjected to any form of therapy.

H Other points of interest

It is worth noting that of 72 cases who were advised to have regular medical checks on the

basis of the findings at the examination in 1962-64 only 42 (68%) had had these checks. The lowest figure was recorded in the intermittent proteinuria group in which only 20 (57%) out of 35 who had been recommended to have checks had followed this advice.

DISCUSSION

It is scarcely possible to conceive of a single definition of the term proteinuria (15) since among other reasons the protein excretion of normal persons varies within wide limits. Thus vigorous physical activity even in persons with healthy kidneys leads to such a great rise in excretion of serum protein in the urine that the common routine tests for proteinuria may be positive (16, 20, 21, 30). Account must therefore also be taken of the test conditions when judging an isolated proteinuria test. There is also conflicting information about the normal daily excretion of protein in the urine under standardised conditions (7, 16, 22, 29).

In recent years Albustix because of its simple procedure has to an ever increasing extent replaced the previously used precipitating reactions (Heller's nitric acid ring test and the sulphosalicylic acid test). There is however disagreement in the literature as to the value of the test (1, 4, 5, 6, 12, 15, 17, 25, 27, 28). With the object of increasing the certainty of proteinuria diagnosis therefore we did not in the present investigation consider proteinuria to be present until both the Albustix reaction and Heller's nitric acid ring test were positive. During the screening in 1962-64 however only Albustix was used. For this reason the diagnosis transient proteinuria

which was based solely upon this test must be regarded as uncertain in this connection. It must also be pointed out that had the primary examination and the follow up study comprised more than three examinations there would undoubtedly have been a certain amount of regrouping among the proteinuria types.

On this account and since previously published articles often lacked a clear definition of the different concepts of proteinuria it is difficult to make any direct comparisons between the studies presented here and earlier investigations. King found in his follow up study that what he called persistent proteinuria seldom disappeared or became intermittent (9). In other investigations however it was found that non-persistent proteinuria could completely disappear but could also sometimes become persistent (2, 9, 10, 18, 19, 26). The results of the present investigation also lend weight to the argument that inconstant proteinuria may disappear for we found that $\frac{4}{5}$ of the cases which at the primary examination had what is here designated transient or intermittent proteinuria after four years showed negative proteinuria findings in three consecutive examinations. The remainder of the cases in both these groups still had proteinuria of type intermittent or transient. Thus none of these cases had at that time become persistent. Of those cases which from the given definition were judged to have persistent proteinuria at the primary examination only 20% still had constant proteinuria at the follow up examination while fully 30% showed negative findings throughout. This could indicate that a number of the cases which were judged to be persistent at the primary examination would actually have proved to be intermittent if more examinations had been performed at that time.

The frequency of pyuria at the screening in the follow up study was only 3.9% but in quantitative sediment at the clinical examination fully 22.5% had pyuria. This figure which perhaps seems rather high should however be compared with the frequency of pyuria (33.5%) which was obtained by the same method at another examination of subjectively healthy women (11).

The frequency of haematuria too (2.3% in screening and 4.5% at follow up) is not different from the frequency obtained in the above men-

tioned study (4.4%). As a comparison Locock's follow up of fixed orthostatic proteinuria may be mentioned in which after five years he found only one case of haematuria among 61 cases investigated (14). Considering that in the literature the frequency of asymptomatic bacteriuria among women is given as 2.2-6.6% (8) and that the bacteriological diagnosis in the present work is based on only one culture the bacteriuria frequency obtained should not be markedly different from the frequency in "normal" material.

The frequency of cases with increased anti-streptolysin titre seems to be remarkably high even taking into account that the investigation was made in the autumn. Since we do not have access to any control material the frequency figures obtained cannot be evaluated.

Of the two cases with persistent proteinuria which displayed increased serum creatinine values of 1.3 and 1.7 mg% respectively one case had normal creatinine clearance and the other had hypertension with nephrosclerosis.

King (10) and Phillipps et al (19) in follow up investigations of young men with persistent proteinuria recorded diastolic hypertension in 57 and 34% respectively. Concerning orthostatic proteinuria King reported a rise in diastolic blood pressure in 15% while Locock could not verify this in his work (14). In the present study we found two cases of hypertension in the persistent proteinuria group but no difference in the mean values for systolic and diastolic blood pressure between the different proteinuria groups.

In the above mentioned investigation King found that of cases still displaying constant proteinuria at the follow up 53% clinically had chronic pyelonephritis (10) while Phillipps et al who in his follow up study of persistent proteinuria performed kidney biopsies found light microscopic glomerular changes in 74% (19). The results of the investigations mentioned are of course difficult to compare with our own. A number of the cases which as a result of the proteinuria findings of 1962-64 were called persistent would for example certainly have been intermittent as was mentioned earlier. The latter situation is in all probability part of the explanation why fully 65% of the cases with persistent proteinuria in 1962-64 did not display clinical signs of urinary tract disease at the check up in 1967. On the other hand the four cases

which still had persistent proteinuria in 1967 did not show signs of kidney disease either

Concerning non persistent forms of proteinuria previous follow up studies which in a number of cases included kidney biopsy have revealed both overt and sub-clinical kidney diseases and also a number of cases of metabolic disturbance (14 17 23 24) In the present check up on the cases displaying transient or intermittent proteinuria at the primary examination in 1962-64 urinary tract disease was found in 6% a frequency which does not seem to differ from that in a "normal material"

To sum up it may be said that for this four year follow up study of cases with different forms of isolated proteinuria the results concerning pathological findings were strikingly modest It must be pointed out however that a more extensive examination including kidney biopsy would in all certainty have revealed an additional number of cases with kidney diseases Bearing in mind the higher mortality for different forms of isolated proteinuria compared with normal material which has been demonstrated by long term observations further follow up studies of the material presented here would be of great interest (3 13) The results of the present study indicate that in cases of transient or intermittent isolated proteinuria there is no medical motivation for too frequent checks which may lead to the patient becoming psychically unbalanced The observation that only $\frac{1}{3}$ of the individuals who had been recommended to have continued medical checks at the primary examination in 1962-64 had taken this advice is remarkable This serious situation which reduces the value of general health surveys should be noted when planning the organisation of similar examinations

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Group C renal autotransplantation with uretero-ureteral anastomosis followed by contralateral nephrectomy.

Conventional technique was used for performance of the nephrectomy in Group A.

The autotransplantation in Group B was commenced by nephrectomy performed so that the renal artery and vein were severed close to the inferior vena cava and aorta, respectively. The ureter was severed while maintaining its vascular supply and ligated at a distance of 10–15 cm from the kidney. After removal the kidney was treated as described in the section dealing with preparation of the kidney. The kidney was reimplanted by end-to-end anastomosis of the renal artery and the external iliac artery and by end-to-side anastomosis of the renal vein and the external iliac vein generally on the right side using 6/0 silk on an atraumatic needle. Re-establishment of the urinary tract was ensured after the ureter was spatulated on the distal 1 cm, by suturing the ureter into the lumen of the bladder and then sewing the wall of the bladder to the adventitia of the ureter. Ureteral anastomosis was done with 5/0 atraumatic catgut. Conventional technique was used for the contralateral nephrectomy.

The autotransplantation in Group C was performed as described for Group B except that the urinary tract was re-established by means of a uretero-ureteral anastomosis. When placing this, the two ends of the ureter were spatulated before suturing with 6/0 atraumatic catgut. Contralateral nephrectomy was performed by the conventional method.

At the end of the operation a million IU benzyl penicillin sodium NF₆ (Penicillin Leo) and 1 g streptomycin NF₆ (Duo-Streptomycin Novo Novojex) were administered into the peritoneal cavity. The abdominal wall was closed in several layers with interrupted sutures of silk. On each of the first four days after the operation the pigs were given 16 000 IU per kg benzyl penicillin sodium NF₆ and 70 mg per kg d-hydrostreptomycin sulfas NF₆ (Streptopropalin Rosco vet 2). The sutures were removed ten days after the operation.

Perfusion of the Kidney

After removal the kidney was immediately perfused with 400 ml cold (4°C) low molecular weight dexsan (average MW 40 000) (10% Rheomacrodex with sodium chloride) containing 2 000 IU heparinum NF₆ (Heparin Leo) and 1 g procaine chloridum NF₆ administered from a flask at a height of about 130 cm. The perfusion time was from 10 to 20 min. The temperature 15 mm inside the kidney was measured in three cases and at the end of the perfusion the average temperature was 13°C (thermistors made by E. L. Laboratories, Copenhagen). Reimplantation was started immediately after cessation of the perfusion, and during stitching the kidney was not cooled externally. Recirculation with blood commenced after 30 to 40 min and the total ischaemic time thus varied from 40 to 70 min.

Post-operative Studies

Blood analyses. During the first week after the operation, blood specimens for the determination of pH, haematocrit and concentrations of creatinine, urea, sodium,

potassium and chloride in plasma were taken every other day and subsequently once a week for the following three months.

Kidney function. Simultaneous determinations were made of the clearance of inulin, endogenous creatinine, urea and para-amino-hippuric acid (PAH) and of the excretion percentages of water, sodium, potassium and chloride. The experiments were made in unanaesthetized animals. The first experiment was done ten days after the operation and the following experiments every 2–3 weeks. Each experiment consisted of at least three periods of 30 min and the last experiment concluded with three periods for the determination of the maximal excretion (T_m) of PAH. For details of the test substances, clearance technique and methods of calculation see Gyrd Hansen (3).

Analytical methods. Inulin (Brun (3)), endogenous creatinine (Bonnes and Tausky (1)), PAH (Bratton and Marshall (2)), urea (Conway (4)), chloride (Schales and Schales (5)). Potassium and sodium were determined by flame photometry (Beckman Direct Reading Flame Photometer). All the plasma concentrations found were calculated to correspond to the value in the water phase of the plasma being assumed to be 8. The pH was determined by a microglass electrode (Radiometer) and the haematocrit values by a microhaematocrit tube after centrifugation in a MSE Micro Haematocrit Centrifuge for 15 min at 4 000 rpm (4 000 \times g).

Post-mortem examination. After the last clearance experiment the animals were killed by shooting and bled, and a post-mortem examination performed immediately. The kidneys were weighed and tissue was removed for histological examination and fixed in buffered formalin. Paraffin wax sections were stained with iron haematoxylin-van Gieson and the periodic acid-Schiff reaction was carried out according to McManus and Murray (14).

RESULTS

Operation was performed on 17 pigs (i.e. five in each of Groups A and C and seven in Group B). Two of the latter died about a month after the operation as a result of ureteral stenosis with the development of hydronephrosis and uraemia and are therefore omitted from the Group B results.

Concentrations of Creatinine, Urea, Sodium, Potassium and Chloride in Plasma

Fig. 1 shows the average concentrations of creatinine (μ g/ml) in plasma in Groups A, B and C during 105 days after the operation.

It will be seen that in Groups B and C the creatinine concentration in the plasma of FF increased to higher levels than in the Group A animals during the first six days after the operation. This difference was statistically significant.

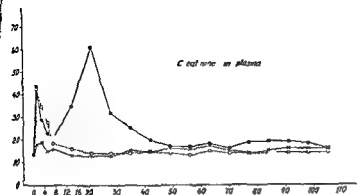


Fig 1 Concentration of creatinine in plasma 0-105 days after operation Ordinate creatinine in plasma $\mu\text{g/ml}$ Abscissa days after operation $\times-\times$ Group A $\bullet-\bullet$ Group B $\circ-\circ$ Group C

on the 1st day ($p < 0.001$). On the 7th day the creatinine concentration in plasma was not significantly different in the three groups. During the remainder of the observation period there was only a slight difference between the creatinine concentration in plasma in Groups A and C ($p > 0.10$). The creatinine concentration in plasma of the Group B pigs increased from the 7th day and reached its maximum on the 21st day. However the standard deviation in Group B was so great that comparison with Group A on the 21st day showed no significant difference ($0.05 < p < 0.1$). From the 50th day the creatinine concentration in plasma in Group B was only slightly higher than in Group A ($p > 0.025$).

Fig 2 shows the concentration of urea in plasma of the pigs in the three groups. It will be seen that the urea content varied during the experimental period exactly in parallel to the creatinine content (Fig 1). The concentration of sodium, potassium and chloride in plasma was constant throughout the experimental period and

was identical in the three groups: viz 143 mEq/l (128-164), 4.4 mEq/l (3.3-5.3) and 109 mEq/l (102-115) respectively.

The average haematocrit varied during the experimental period from 33 to 42. The lowest values were observed in Group B during the first four weeks after the operation (Tables I, II and III).

The pH of the blood was quite constant during the experimental period and was the same for the three groups with an average of 7.39 ± 0.06 .

Clearance of Inulin, Endogenous Creatinine, Urea and PAH

Tables I, II and III show the average values for Groups A, B and C. In Group A the clearances as well as total renal plasma flow ($\text{RPF}_{\text{total}}$) and total renal blood flow ($\text{RBF}_{\text{total}}$) remained constant during the experimental period.

In Group B clearance as well as $\text{RPF}_{\text{total}}$ and

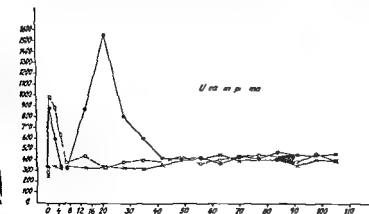


Fig 2 Concentration of urea in plasma 0-105 days after operation Ordinate urea in plasma $\mu\text{g/ml}$ Abscissa days after operation $\times-\times$ Group A $\bullet-\bullet$ Group B $\circ-\circ$ Group C

Table I Average renal clearances in five pigs 10 to 94 days after unilateral nephrectomy Group A

The bracketed figures indicate minimal and maximal values of single experiments

Days after nephrectomy	Body weight (kg)	Clearance					
		Haematocrit (%)	Diuresis (ml/min)	Inulin (ml/min 10 kg b wt.)	Endogen. creatinine (ml/min 10 kg b wt.)	Urea (ml/min 10 kg b wt.)	PAH (ml/min kg b wt.)
10	5 (4-6)	38 (35-41)	0.8 (0.7-0.9)	16 (14-19)	16 (12-20)	8 (7-10)	52 (44-60)
31	75 (70-79)	40 (37-41)	1.3 (1.1-1.5)	17 (15-19)	18 (16-20)	9 (8-11)	53 (45-60)
50	90 (83-96)	41 (40-43)	1.7 (1.4-2.0)	17 (15-19)	17 (16-19)	9 (5-10)	55 (41-61)
73	106 (100-112)	39 (35-43)	2.2 (1.8-2.6)	17 (15-20)	17 (16-19)	10 (9-11)	55 (41-61)
94	117 (111-126)	42 (39-44)	2.2 (1.5-3.0)	17 (15-17)	17 (16-19)	10 (9-11)	57 (50-61)

Table II Average renal clearances in five pigs 10 to 94 days after bilateral nephrectomy and unilateral auto-transplantation Uretero-vesical anastomosis Group B

The bracketed figures indicate minimal and maximal values of single experiments

Days after transplan- tation	Body weight (kg)	Clearance					
		Haematocrit (%)	Diuresis (ml/min)	Inulin (ml/min 10 kg b wt.)	Endogen. creatinine (ml/min 10 kg b wt.)	Urea (ml/min 10 kg b wt.)	PAH (ml/min kg b wt.)
10	60 (49-67)	35 (27-42)	1.9 (0.7-5.4)	10 (7-15)	12 (7-16)	6 (3-9)	3 (1-5)
1	67 (57-6)	35 (33-47)	3.9 (1.3-8.0)	11 (7-22)	12 (8-21)	7 (4-11)	4 (2-6)
49	88 (72-96)	38 (34-47)	2.5 (1.9-3.1)	14 (11-19)	17 (11-21)	9 (7-12)	41 (30-61)
73	99 (85-104)	41 (39-43)	3.1 (1.6-6.4)	15 (11-19)	18 (13-22)	9 (7-10)	51 (40-61)
94	113 (97-122)	40 (38-43)	3.4 (1.9-5.3)	15 (11-20)	15 (11-20)	9 (7-13)	40 (33-61)

Table III Average renal clearances in five pigs 10 to 94 days after bilateral nephrectomy and unilateral auto-transplantation Uretero-uterine anastomosis Group C

The bracketed figures indicate minimal and maximal values of single experiments

Days after transplan- tation	Body weight (kg)	Clearance					
		Haematocrit (%)	Diuresis (ml/min)	Inulin (ml/min 10 kg b wt.)	Endogen. creatinine (ml/min 10 kg b wt.)	Urea (ml/min 10 kg b wt.)	PAH (ml/min kg b wt.)
10	34 (23-44)	33 (24-39)	2.0 (0.8-3)	13 (7-19)	15 (9-22)	7 (4-10)	47 (5-61)
31	72 (65-84)	38 (35-41)	1.7 (1.2-2.3)	19 (17-23)	19 (18-20)	11 (10-11)	69 (61-71)
52	85 (77-97)	38 (34-47)	1.7 (0.9-2)	19 (18-21)	20 (18-21)	10 (9-17)	70 (61-71)
73	105 (100-112)	40 (38-43)	3.0 (2.5-3.9)	18 (17-19)	20 (19-21)	17 (11-13)	61 (48-61)
94	120 (111-124)	40 (38-44)	2.6 (2.3-2.8)	18 (17-19)	18 (16-19)	10 (9-13)	67 (5-61)

RBF_{total} increased until 73 days after the transplantation and then became stabilized.

In Group C clearances as well as RPF_{total} and RBF_{total} increased until the 31st day and then became stabilized. In calculating the total renal plasma flow an extraction percentage of 87 was used, as found by Gyrd-Hansen (9) in normal pigs.

Since the experimental animals were immediately after the last clearance experiment it was possible to state the clearances both, 10 kg body weight and per 100 g kidney tissue for the final measurements. The average renal clearances for Groups A, B and C are shown in Table IV which also gives the corresponding values for normal pigs (9).

Excretion of Water Sodium Potassium and Chloride

The excretions of water sodium, potassium and chloride for pigs in Groups A, B and C are shown in Tables VI VII and VIII. These values were quite constant from the 10th to the 94th day in each group but for both water and electrolytes the values were higher in Groups II and C than in Group A.

Postmortem Examination

Macroscopical findings

In the unilaterally nephrectomized pigs (Group A) the kidney had normal colour. The kidney weights are shown in Table IX; the average weight was 0.26% of the body weight.

In Group B the kidneys from pigs 16, 26, 27 and 28 were normal in colour but firm. In pig 18 one pole of the kidney was completely atrophied and the remaining tissue had a normal colour but was very firm. In pigs 16, 18, 27 and 28 the papillae were strongly flattened and the pelvis and ureter were greatly distended. There was a swelling at the entry of the ureter into the bladder which caused a severe stenosis of the distal part of the ureter. In the kidney from pig 26 the papillae and pelvis were normal and the ureter only slightly distended. There was only a little narrowing around the opening to the bladder. The relative kidney weights of the pigs in Group B are shown in Table IX; the average weight was 0.32% of the body weight.

As regards Group C the kidneys were of normal colour and were slightly firm. The papillae and pelvis were normal. There was a slight narrowing at the ureter anastomosis, and proximally to the anastomosis the ureter was only slightly dilated. The relative kidney weights are shown in Table IX; the average weight was 0.37% of the body weight.

In all the pigs both the arterial and venous vascular anastomosis healed without any sign of stenosis or thrombus formation.

Microscopical findings

Apart from slight interstitial fibrosis in the kidney from pig 23 the kidneys from the Group A animals were normal.

In Group B pig 18 had chronic purulent pyelonephritis comprising about 10% of the kidney.

Total renal plasma flow (ml/min/10 kg b wt.)	Total renal blood flow (ml/min/10 kg b wt.)	Clearance ratios		
		Cr In	Urea In	Filtration fraction In PAH
65 (60-75)	105 (94-121)	1.0	0.5	0.31
66 (56-86)	109 (89-143)	1.1	0.5	0.31
69 (60-76)	117 (105-131)	1.0	0.5	0.31
88 (59-92)	114 (95-156)	1.0	0.6	0.32
72 (6-101)	123 (107-171)	1.0	0.6	0.34

Total renal plasma flow (ml/min/10 kg b wt.)	Total renal blood flow (ml/min/10 kg b wt.)	Clearance ratios		
		Cr In	Urea In	Filtration fraction In PAH
41 (26-63)	111 (36-98)	1.2	0.6	0.31
53 (33-94)	84 (47-16)	1.0	0.6	0.29
54 (38-83)	88 (63-143)	1.2	0.6	0.31
65 (30-93)	111 (84-163)	1.2	0.6	0.29
63 (41-90)	105 (67-150)	1.0	0.6	0.30

Total renal plasma flow (ml/min/10 kg b wt.)	Total renal blood flow (ml/min/10 kg b wt.)	Clearance ratios		
		Cr In	Urea In	Filtration fraction In PAH
59 (31-106)	106 (47-140)	1.2	0.5	0.28
111 (76-101)	138 (129-158)	1.0	0.6	0.28
111 (76-96)	142 (115-166)	1.1	0.5	0.3
78 (60-89)	130 (97-143)	1.1	0.7	0.29
84 (69-99)	140 (119-160)	1.0	0.6	0.27

Maximal Tubular Excretion of PAH

The T_m of PAH was determined in the last experiment and the results are shown in Table V for each pig separately together with the plasma concentrations of PAH on which the T_m determinations were made. The T_m values are given both per 10 kg body weight and per 100 g kidney tissue.

Table IV Average renal clearances in pigs 94 to 115 days after operation

Group	Clearance							
	Inulin		Endogenous creatinine		Urea		PAH	
	(ml min ⁻¹ 10 kg b wt.)	(ml min ⁻¹ 100 g kidney)	(ml min ⁻¹ 10 kg b wt.)	(ml min ⁻¹ 100 g kidney)	(ml min ⁻¹ 10 kg b wt.)	(ml min ⁻¹ 100 g kidney)	(ml min ⁻¹ 10 kg b wt.)	(ml min ⁻¹ 100 g kidney)
A	16	59	16	61	10	36	56	209
B	14	46	16	49	9	27	54	171
C	18	49	18	48	10	29	67	187
Normal pigs	21	56	22	61	12	34	64	174

Gyrd Hansen 1968 (9)

tissue. The kidneys from pigs 16, 27 and 28 showed focal interstitial nephritis and interstitial fibrosis which was most pronounced around the glomeruli and vessels as well as in the medulla. The kidney from pig 26 was normal.

In Group C the kidneys from three pigs (nos 30, 33 and 39) showed a slight degree of diffuse interstitial fibrosis. There was focal intense scar like connective tissue in the renal cortex in 35 while the kidney from pig 37 was normal.

DISCUSSION

The concentration of creatinine and urea in plasma (Figs 1 and 2) was within normal limits

for the nephrectomized pigs (Group A) during the whole observation period. According to Gyrd Hansen (9) the normal values are 7 to 19 µg/ml for creatinine and 240–590 µg/ml for urea. In contrast the concentration of creatinine and urea increased immediately after autotransplantation by both techniques and then became normal within a week. A similar increase in the urea concentration in plasma has been seen after autotransplantation in dogs (7) and homotransplantation in man (24). The increase in the creatinine and urea concentration in plasma observed from the 2nd to the 7th week in the Group B animals was probably due to transient stenosis at the uretero-vesical anastomosis.

Table V Clearance and maximal tubular excretion (Tm) of para-aminohippuric acid 94 to 115 days after operation

Group	Pig no.	Body weight (kg)	Kidney weight (g)	Plasma concentration of PAH (µg/ml)	PAH clearance (ml min ⁻¹ 10 kg b wt.)	Inulin clearance (ml min ⁻¹ 10 kg b wt.)	Tm (mg min ⁻¹ 10 kg b wt.)	Tm (mg min ⁻¹ 100 g kidney)
A	23	177	265	10.0	6	1	18	6
	25	115	305	1090	31	13	19	70
	37	140	400	1600	4	11	1	7
	39	111	304	1490	23	12	70	73
Average							19	3
B	26	117	395	710	41	11	19	4
	27	130	450	930	34	14	19	54
	28	136	400	1110	78	13	70	69
Average							19	60
C	30	118	310	1870	26	11	21	70
	33	133	450	950	49	15	33	87
	35	111	407	1000	39	15	24	67
	36	144	457	140	34	13	6	6
	37	122	410	115	31	13	1	70
Average							5	70

Table VI Renal excretion of water and electrolytes in five pigs 10 to 94 days after unilateral nephrectomy

Days after nephrectomy	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	58	0.8	16	0.83	0.04	11.0	0.38
31	75	1.3	17	0.97	0.07	16.5	0.24
52	90	1.7	17	1.11	0.02	22.0	0.24
73	106	2.2	17	1.22	0.11	19.0	0.48
94	117	2.2	17	1.13	0.08	17.7	0.41
Average			17	1.05	0.05	17.2	0.35

Table VII Renal excretion of water and electrolytes in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero vesical anastomosis

Days after transplantation	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	60	1.9	10	3.18	0.30	29.0	1.10
31	67	3.9	12	4.85	0.48	37.4	1.75
52	85	2.5	14	2.09	0.12	25.0	1.27
73	98	3.1	15	2.11	0.23	24.7	0.93
94	113	3.4	15	2.01	0.21	30.0	1.00
Average			13	2.85	0.27	29.2	1.21

Table VIII Renal excretion of water and electrolytes in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero ureteral anastomosis

Days after transplantation	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	58	2.0	13	2.84	0.38	32.0	1.26
31	74	1.7	19	1.24	0.05	30.6	0.84
52	85	1.7	19	1.05	0.02	14.2	0.37
73	105	3.0	18	1.59	0.17	30.6	0.89
94	120	2.6	18	1.22	0.22	20.0	0.72
Average			17	1.59	0.17	25.5	0.82

Table IX Relative kidney weight in pigs 94 to 115 days after operation

Group	A					B					C				
Pig no	20	21	23	32	39	16	18	26	27	31	30	33	35	36	37
Body weight kg	1.5	1.27	1.15	1.40	1.11	1.15	1.15	1.17	1.30	1.36	1.18	1.23	1.11	1.24	1.22
Kidney weight g	353	265	305	400	304	315	375	395	450	400	370	450	402	557	410
Relative kidney weight	0.28	0.21	0.27	0.29	0.26	0.27	0.33	0.34	0.35	0.29	0.31	0.37	0.36	0.45	0.34

Table X. Comparison between the clearances in normal pigs and in unilaterally nephrectomized pigs

Days after nephrectomy	<i>p</i> -values for the difference between the clearances of			
	Inulin	Creatinine	Urea	PAH
10	$p < 0.001$	$0.01 < p < 0.02$	$0.001 < p < 0.005$	$0.05 < p < 0.10$
31	$p < 0.001$	$0.01 < p < 0.02$	$0.01 < p < 0.02$	$0.10 < p < 0.20$
52	$p < 0.001$	$0.025 < p < 0.05$	$0.01 < p < 0.02$	$0.10 < p < 0.20$
73	$0.001 < p < 0.005$	$0.025 < p < 0.05$	$0.05 < p < 0.10$	$0.05 < p < 0.10$
94	$p < 0.001$	$0.025 < p < 0.05$	$0.05 < p < 0.10$	$0.30 < p < 0.40$

Comparison of the mean, endogenous creatinine, urea and PAH clearances in unilaterally nephrectomized pigs with the corresponding clearances in normal pigs with two kidneys (9) shows that the average clearance in the former group during the 94 days of observation was 80–85% of that of normal pigs. The clearance values in nephrectomized dogs (16, 19, 14) and man (23, 6, 18) have been found to be reduced to 60 to 70%. The high compensatory hyperfunction found in the present study is probably due to the fact that the nephrectomy was performed on young animals. Ogden (18) found that the ability to develop compensatory hyperfunction is greater in young individuals.

Statistical evaluation of the means and standard deviations for nephrectomized and normal pigs gave the *p* values (*t*-test) shown in Table X for the differences 10, 31, 52, 73 and 94 days after nephrectomy. In all cases the means are lower in the nephrectomized than in the normal pigs, but a significant difference (at the 0.01 level) was found only for inulin clearance and for urea clearance on the 10th day.

The clearance of inulin, endogenous creatinine, urea and PAH were lower in the Group II animals (autotransplantation and uretero-ureteral anastomosis) than in nephrectomized pigs. This difference was most pronounced at the examinations on the 10th and 31st days after the operation (Table II), i.e. in the period during which an increased concentration of creatinine in the plasma was observed (Figs. 1 and 2). The results in Group B show large standard deviations, presumably on account of the varying degree of ureteral anastomosis. No statistically significant difference (at the 0.01 level) could be demonstrated between Group B and the nephrectomized pigs.

The renal clearances in Group C (autotrans-

plantation and uretero-ureteral anastomosis) in which there were no surgical complications after the operation, were about the same on the 10th day after the operation as those of the nephrectomized pigs. From the 31st day the average clearance values for Group C were higher than those of the nephrectomized animals. This tendency was most marked from the 31st to 73rd day and the difference on the 52nd day was statistically significant ($p < 0.01$) for creatinine and PAH. On the 94th day the difference between the average clearance values for Groups A and C was less ($0.1 < p < 0.2$). Thus, the renal clearance in Group C corresponds to that of the autotransplanted kidneys in dogs (5, 17, 7) and homotransplanted kidneys in man (11, 22, 10, 18) if the organ shows no signs of rejection.

The maximal tubular excretion of PAH both for nephrectomized and autotransplanted pigs (Table VIII) was within the variation found for normal pigs (23 (± 6) mg/min/10 kg body weight (9)). Similar results were found in nephrectomized dogs (see 13, 14).

Calculated per 10 kg body weight, the average *Tm* for Group C was greater than for Group A ($0.025 < p < 0.05$) while per 100 g kidney tissue it was the same for the two groups ($0.60 < p < 0.70$). This is in complete agreement with the fact that the relative kidney weights in Group C (0.37) were greater than in Group A (0.26) (Table IX) ($0.001 < p < 0.005$). The relative kidney weights in Group C were identical with those of normal pigs with two kidneys (0.36) ($0.60 < p < 0.70$) while they were only about 70% in nephrectomized pigs. This degree of hypertrophy in unilaterally nephrectomized pigs corresponds to the hypertrophy found in unilaterally nephrectomized dogs (20).

Excretion of water was two to five times

higher in Group II pigs than in the nephrectomized animals while in Group C it was one to three times higher. The excretion of electrolytes was greater in the autotransplanted than in the nephrectomized pigs which is probably due to the denervating of the transplanted kidney (12) or to damage to the tissue occurring during the ischaemic period.

The ureteral stenosis observed and the consequent hydronephrosis together with the histological findings in Group II explain the lower clearances found in that group. The slight changes demonstrated at histological examination of the kidneys from Group C have had no effect on the renal clearance. These correspond to the changes found by Dempster et al (5), Murray et al (17) and Fontaine (8) in the autotransplanted kidneys of dogs.

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RENAL EXCRETION OF LOW AND HIGH MOLECULAR WEIGHT POLYVINYLPYRROLIDONE (PVP) IN PATIENTS WITH PROTEINURIA

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Abstract The renal excretion of two types of PVP with low and high molecular weight (LMW PVP and HMW PVP) has been investigated in normal subjects and in patients with proteinuria due to primary renal disease. A significant excretion was found also of HMW PVP.

The patients with proteinuria had a slower excretion of PVP than the control subjects. During the first hours after injection the quantity of PVP in the urine was much less in the patients. This quantity correlated positively with the creatinine clearance.

It seems probable that initially the smaller molecules of the PVP sample are excreted and that this excretion is mainly dependent upon the GFR. In a later phase excretion in the patients was on a relatively higher level than in the controls. This may be due both to increased glomerular permeability for the larger PVP molecules and to a longer serum half life of PVP.

Protein clearances were carried out concurrently which made it possible to divide the patients into a selective and a non selective type of proteinuria. The mean ratio LMW PVP/HMW PVP in the non-selective group was different from the selective group and from the control group. No difference was found in PVP permeability between the selective group and the control group. It is concluded that this PVP ratio may demonstrate differences in glomerular permeability between groups of patients. However with the testing material used in this study the overlap is too large for determination of selectivity in individual cases.

In recent years glomerular permeability in patients with the nephrotic syndrome has been investigated by clearance studies of serum proteins. The selectivity of protein excretion which can be concluded from these clearances is of significant clinical value (3 5 6 7 11 13 14). Apparently there is a correlation between the change in glomerular permeability and the molecular weight distribution of urinary proteins. Theoretical objections however to the use of proteins for

permeability studies are the varying shape and charge of protein molecules and the possibility of excretion as subunits or complexes.

Another approach to glomerular permeability studies can be found in the use of inert macromolecules like dextran (1 4) or polyvinylpyrrolidone (PVP). Recently Hulme and Hardwicke (10) estimated PVP clearances in patients with the nephrotic syndrome as an index of selectivity.

The purpose of this study was to investigate the renal excretion of two types of PVP with low and high molecular weight respectively (LMW PVP and HMW PVP) in normal subjects and patients with glomerular proteinuria. A ratio of excreted LMW PVP and HMW PVP was assessed and compared with selectivity patterns as estimated by an immunological method.

MATERIAL AND METHODS

All patients had proteinuria due to primary renal disease. Relevant data concerning renal biopsy diagnosis by light microscopy degree of proteinuria immunologically determined selectivity and creatinine clearance are given in Table I. Criteria for histological diagnosis were the same as described in earlier studies from this Department (13 14).

Two series of experiments were arranged. In the first series 20 patients (group A nos 1-20 in Table I) were studied during hospitalization. Numbers 1-20 in Table I correspond to numbers 1-20 in Table I of the previous article (9) where further laboratory data were presented. Simultaneously 19 normal control subjects matched for sex and age and without any symptom of renal disease were studied.

In the second series 11 patients (group B) were studied in the Outpatient Department. This group comprised both patients already studied in the first series (nos 7 13

Table 1 Laboratory data renal biopsy diagnosis and urinary PVP excretion in 25 patients with proteinuria due to primary renal disease

Excreted amounts of PVP in urine (of injected dose)												
Pat no	Age (y)	Sex	Biopsy diagnosis	Proteinuria (g/24 h)	Selective (S) or non selective (N)	Creatinine clearance	Group A 24 hour excretion				Group B 3 hour excretion	
							LMW PVP	HMW-PVP	Ratio LMW-PVP/HMW-PVP	LMW-PVP	LMW-PVP	
1	49	♂	Membr glom neph	1.1	N	55	21.5	22.2	0.97			
2	57	♂	Focal focal glom neph	0.9	N	69	41.6	22.1	1.88			
3	30	♂	Membr glom neph	4.3	N	51	47.7	19.2	2.22			
4	18	♂	Membr glom neph	0.7	S	116	42.8	18.3	2.33			
5	66	♂	Focal focal glom neph	3.5	N	54	35.9	14.9	2.41			
6	7	♂	Minimal lesions	0.3	S	120	75.7	25.6	3.96			
7	18	♂	Prolif glom neph	70.2	N	15	27.7	17.7	1.56		9.6	
8	15	♂	Membr glom neph	10.1	N	104	27.0	16.4	1.65			
9	39	♂	Membr glom neph	2.9	N	86	30.5	20.5	1.48			
10	42	♂	Membr prolifer glom neph	2.7	N	99	21.0	14.3	1.47			
11	45	♂	Membr glom neph	1.4	N	144	12.0	8.1	1.48			
12	12	♂	Focal focal glom neph	0.7	S	—	35.9	20.2	1.78		24.7	
13	15	♂	Prolif glom neph	5.8	N	72	16.8	14.3	1.17			
14	34	♂	Membr glom neph	4.4	N	77	15.0	10.8	1.38			
15	39	♂	Prolif glom neph	3.4	N	62	46.1	17.4	1.31		26.4	
16	73	♂	Membr glom neph	1.8	S	35	20.8	11.8	1.76			
17	19	♂	Membr glom neph	8.6	N	140	17.7	15.9	1.12		32.2	
18	51	♂	Minimal lesions	1.1	S	92	12.2	15.2	0.80			
19	67	♂	Membr prolifer glom neph	7.7	N	75	14.1	7.8	1.80		13.4	
20	37	♂	Minimal lesions	16.3	S	91	41.0	16.5	2.48		22.4	
21	16	♂	Focal focal glom neph	4.4	N	25					13.0	
22	18	♂	Focal focal glom neph	4.7	N	8					19.0	
23	21	♂	Prolif glom neph	6.8	N	30					16.9	
24	24	♂	Lobular glom neph	8.8	N	60					4.4	
25	36	+	Membr prolifer glom neph	5.5	N	60					21.0	

15 17 19 20) and patients not studied before (nos 21-25) Concurrently with group B 11 control subjects again matched for sex and age were studied

Two types of PVP of a different molecular weight distribution were obtained in pyrogen free sterile isotonic solutions from the Radiochemical Centre Amersham England The mean molecular weights were reported as 40 000 (low molecular weight LMW PVP) and 160 000 (high molecular weight, HMW PVP) respectively Data of analytical ultracentrifuge and gel filtration studies were given in the previous article (9)

For studies in patients of group A and their controls the LMW PVP was labelled with ^{125}I and the HMW PVP with ^{131}I In all cases 25 μC of each type was injected intravenously The two types of PVP were mixed shortly before injection Urine was collected during 24 hours after injection In some patients and controls urine was collected over three consecutive periods on the first day (0-3 3-10 10-24 hours) and in others urinary excretion was also estimated on the second and third day after injection

For studies in patients of group B and their controls another batch of LMW PVP was used which was labelled with ^{125}I Again 25 μC was injected intravenously Urine was collected during the first three hours after injection

In all patients thyroid uptake was blocked with Lugol's solution

The amount of radioactivity was determined as described in the previous article (9) and expressed as a percentage of the injected dose The amount of urinary protein was estimated by the biuret method The reported data (Table I) are the mean of the excreted protein on four consecutive days except in patients 21-25 for whom a one-day value is given There was a considerable variation in urinary protein loss between the patients Patients with a low degree of proteinuria at the time of the present study had a higher excretion at earlier observations but immunologically determined selectivity patterns which may be inaccurate in cases with slight proteinuria, were essentially unchanged Protein clearances in group A were carried out twice during the clinical admission The method was the same as described earlier from this Department (13 14) and essentially similar to the one introduced by Blainey et al (3) Excretion patterns were defined as selective when the IgG clearance was below 70% of the transferrin clearance and both alpha₂ macroglobulin and alpha₁ lipoprotein were immunologically undetectable in non-concentrated urine samples

Serum creatinine was estimated as mentioned in the previous article (9)

In this study it appeared unnecessary to take into account the quantity of free iodine excreted in urine Both in patients and control subjects the proportion of free iodine averaged 7% of the total excreted radioactivity This was valid for both the LMW PVP and the HMW PVP Free iodine was estimated with an ion exchange resin as well as with dialysis No differences of any importance were found between the ratios LMW PVP/HMW PVP calculated with values corrected for free iodine as compared to non-corrected values

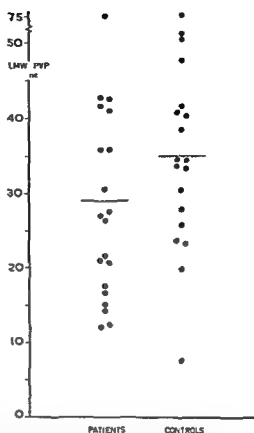


Fig 1 Urinary excretion of LMW PVP during 24 hours after injection in patients of group A and their controls Amounts of PVP are expressed as percentages of the injected dose

RESULTS

Fig 1 summarizes data on the urinary excretion of LMW PVP during the first 24 hours after injection in the patients of group A and their controls In both groups a considerable part of the injected radioactivity is excreted in the urine during this time However in the patient group this quantity is on the average significantly lower than in the control group ($p < 0.05$)

In Fig 2 comparable results for the excretion of HMW PVP are shown Again a substantial although smaller amount was excreted on the first day after injection and likewise this was lower in the patient group ($p < 0.025$)

In seven patients (nos 13-20) and in six control subjects the 24 hour urine collection was divided into three consecutive samples The results are shown in Fig 3 for LMW PVP and in Fig 4

It may be concluded that the permeability in the non selective patient group is different from normal and from the selective group but that there is no difference in permeability between the selective patients and normal subjects.

This method therefore may demonstrate differences in permeability between patient groups. With the PVP batches available for this study there was some overlap between ratios in selective and non selective proteinurias. In individual cases no definite conclusion can be drawn as regards selectivity. Probably better results can be expected with very sharp peaks of LMW PVP and HMW PVP without any overlap between the molecular weight ranges of the two types.

Because this method can be used to investigate capillary transfer mechanisms in other tissues and organ systems such as the gastro-intestinal tract in which immunological protein clearances are difficult to perform for technical reasons a wider use seems possible.

ACKNOWLEDGEMENT

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GLOMERULONEPHRITIS WITH INITIAL LUNG PURPURA (GOODPASTURE'S SYNDROME)

Survival of Two Patients out of Four

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Abstract Four cases of glomerulonephritis with initial lung purpura (Goodpasture's syndrome) are presented. Two patients succumbed to their disease after a few months one being treated with corticosteroids and hemodialysis, the other with corticosteroids only. The third patient showed complete reversal of both the pulmonary and the renal symptoms 10 months after onset of the disease. He received no chemotherapy. Now he has been in full time occupation for more than six months. The fourth patient, during a period of five years, experienced three episodes of glomerulonephritis with initial pulmonary hemorrhage. The symptoms, especially the hemoptysis, were far more pronounced during the first episode and seemed to respond favorably to corticosteroid therapy. No chemotherapy was given during the last episode. For the last 3-4 years the patient has been in good health. Patients with severe pulmonary hemorrhage and minor renal symptoms may respond favorably to corticosteroid therapy. The grave prognosis in Goodpasture's syndrome is based primarily on autopsy series. Benign forms do exist and should not be overlooked or left unpublished.

Glomerulonephritis with initial lung purpura or Goodpasture's syndrome is usually a disease of white men clinically characterized by an insidious course with hemoptysis, anemia, proteinuria, hematuria and increasing dyspnea and azotemia. The disease is usually fatal within a few weeks or months, the patients dying of renal failure or massive pulmonary hemorrhage (2, 5, 8). Pathologically the lungs show multiple hemorrhages with hemosiderosis, thickening of alveolar septa and numerous hemosiderophores in the alveoli. The kidneys give a picture of glomerulonephritis with proliferative changes or hyalinization and fibrosis in more long lasting cases. The etiological factors are still unknown but the consistent clinical and pathological findings establish

Goodpasture's syndrome as a specific disease entity distinct from other disorders involving the kidneys and lungs such as periarteritis nodosa, Wegener's granulomatosis, lupus erythematosus disseminatus and idiopathic pulmonary hemosiderosis (2, 5, 8).

The therapeutic regimens include corticosteroids, hemodialysis, bilateral nephrectomy with intermittent hemodialysis or renal transplantation. However, the number of patients reported to have survived the acute illness is only about 25 (1-4, 6-11, 13-21), the observation period in several cases being only some months.

This paper presents four additional patients with glomerulonephritis and initial lung purpura, two of whom have survived.

CASE REPORTS

Case 1

A 51-year-old quay foreman and previous prize fighter was admitted to hospital on 13 December 1967. He had always been in good health, and annual routine examination findings had been negative. He gave a history of common cold, coughing, hemoptysis, fever and weakness for two weeks and macroscopic hematuria for three days.

He was in good condition but slightly orthopneic. The temperature was 39°C and the blood pressure 140/90 mm Hg. There were crepitations over both sides of the lower chest. Ophthalmoscopic findings were normal except for a small degenerative spot in the right choroid.

Laboratory data were: urine specific gravity 1028, proteinuria (0.3) marked hematuria and a few granular casts. Hb 13.9 g/100 ml, red blood cell count 5.2 mill/mm, MCHC 30%, white blood cell count 15,600/mm³ with normal differential count, reticulocytes 1.5%, ESR 104 mm/h, serum iron 45 µg/100 ml, blood urea 32 mg/100 ml, serum creatinine 0.9 mg/100 ml. Bleeding

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HAEMODYNAMIC EFFECTS OF PROPRANOLOL AND NITROGLYCERIN IN NORMAL SUBJECTS DURING SUBMAXIMAL EXERCISE

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Abstract The haemodynamic effects of propranolol and nitroglycerin have been studied in four normal subjects during submaximal exercise in the sitting position. The rise in heart rate normally seen after nitroglycerin was prevented by propranolol and the reduction in the cardiac output and mean pressure caused by both drugs together was of the same order as that produced by only one of them.

Nitroglycerin and related compounds have been the principal drugs in the treatment of angina pectoris for about a century. In the last five years, however, substances with a completely different mode of action, β adrenergic blockade, have come to play an increasing role in the therapy of angina pectoris (3, 4, 9, 10, 11, 12, 13, 16, 18, 19).

The combination of these two types of drug, nitrates and β blocking agents, has naturally also been widely used and not only an additive but also a synergistic effect has been reported (1, 14, 17). The promising results of the combined therapy have been attributed to the fact that both types of drugs reduce the work of the heart, nitrates by diminishing peripheral resistance and venous return and β blockers by reducing the force and rate of the heart (17).

In the above mentioned investigations with the combined therapy the haemodynamic studies were however confined to the heart rate (14, 17) or the heart rate and the blood pressure (1). It might be expected that the combined use of nitroglycerin and β blockers would cause a precipitous fall in cardiac output. In the present study on a few normal subjects some preliminary observations are therefore reported on the haemodynamic effects of propranolol and nitroglycerin when used alone and together during submaximal exercise.

METHODS

Four healthy male volunteers, aged 25, 39 and 51 years were studied. The subjects were fasting at the examination, which started in the morning. Polythene tubes were inserted in the brachial artery of one arm and in a cubital vein of the other. (2) Measurement of the cardiac output was made by a dye-dilution technique using bromsulphthalein as the indicator. Systemic arterial pressures were registered by means of inductance manometers and recorded to either with the electrocardiogram on a direct writing electrocardiograph (AB Elema Stockholm, Sweden). Peripheral resistance was calculated as the quotient of the mean arterial pressure in mm Hg and the cardiac output in l/min.

The subjects were studied in the supine position at rest and in the sitting position cycling on an electrically braked ergometer (AB Elema). They were first studied without propranolol at rest and during a graded submaximal exercise working six min on each work load (300, 600 or 900 kpm/min; one subject only 300 kpm/min). After six min on the highest work load when the cardiac output had been measured they were given nitroglycerin (0.5 mg sublingually (Dumex)) and after another four to six min of continuous exercise on the same load new haemodynamic measurements were made. After ten min rest 30 mg propranolol (Inderal) was given per os and the subjects were allowed to rest for another 60 min. They were then again examined at rest and during corresponding work loads, a run with nitroglycerin on the highest load.

RESULTS

The haemodynamic effects of propranolol alone are given in Table I. The heart rate was reduced both at rest and during exercise in all four subjects. Also the stroke volume was lowered in most cases so that the cardiac output was reduced correspondingly more than the heart rate both at rest and at work except in one subject (case 3).

Table 1 Haemodynamic data obtained at rest and during exercise in situ position before and after propranolol

Case no	Age	Work load (kpm/min)	Cardiac output (l/min)		Heart rate (beats/min)		Stroke volume (ml)		Pressures (mm Hg)			Peripheral resistance (U)		
			Control	Prop	Control	Prop	Control	Prop	Control	Systol	Diastol	Mean	Control	Prop
1	51	Rest	6.8	5.9	63	56	108	105	114	69	65	81	13.5	13.7
		300	10.5	8.8	81	78	150	113	134	75	68	91	9.2	10.3
2	18	Rest	9.1	6.7	83	68	110	99	176	73	8	83	9.6	12.4
		300	14.6	9.5	132	102	111	93	153	87	109	131	83	7.5
3	39	600	16.4	9.9	168	124	98	80	149	82	108	128	78	104
		Rest	5.1	5.3	70	63	73	84	113	65	84	96	59	72
4	25	100	—	—	102	93	—	—	141	78	102	114	—	—
		600	14.1	11.0	132	110	107	100	157	80	112	128	70	96
		Rest	7.9	7	77	77	102	99	125	70	92	119	91	11.6
		600	14.3	11.1	117	102	122	109	149	75	100	141	78	101
		900	18.4	16.4	140	140	131	137	151	73	78	107	5.8	6.5

Table 1) who at rest had 5.1 l/min before and 5.3 l/min after propranolol. The systolic pressure decreased in practically all cases in the resting as well as the working condition. The effect on the diastolic and mean pressures was more varied although in most cases there was a slight decrease. As the cardiac output decreased and the mean arterial pressure as a whole was unchanged the calculated peripheral resistance rose in most instances.

The circulatory effects of nitroglycerin given during exercise before β blockade are presented in Tables II–VI. They are mainly an increase in heart rate and a decrease in cardiac output, stroke volume and mean pressure (5, 6, 15). It has earlier been found (15) that nitroglycerin given twice with 60 min interval is equally effective implying that it is relevant in this study to compare the effect caused by nitroglycerin before and after propranolol was given (Tables II–VI). As also propranolol reduced the cardiac output, it was interesting to see whether these two substances had synergistic effects but when nitroglycerin was given to subjects under β blockade there seemed to be no further decline of the cardiac output. What happened was that the increase in heart frequency normally found after nitroglycerin was prevented. There was a slight rise in heart rate from the propranolol value but not at all to the same extent as before β blockade. This effect of β blockade has been reported earlier (1, 14, 17). When nitroglycerin was given under β blockade the stroke volume did not decrease to the same degree as before propranolol but the decline of the mean pressure was of the same order as before the β adrenergic blocking agent was given. The increase in peripheral resistance found during propranolol was reduced in two of the four cases by nitroglycerin; in one of them it decreased even below the value found before propranolol.

COMMENTS

The present observations have confirmed earlier reports on the effects of β blockade during exercise (7, 8) apart from the finding by Epstein et al. (8) that there was no consistent alteration of the systemic vascular resistance which in the present study was found to increase in most instances.

Table II *Changes in heart rate (beats/min) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	111	- 3	+21	+ 5
2	600	168	-44	+12	-30
3	600	132	-22	+23	-11
4	900	140	-20	+19	- 2

Table III *Changes in cardiac output (l/min) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	10.5	-1.7	-2.5	-2.4
2	600	16.4	-6.5	-2.0	-3.6
3	600	14.1	-3.1	-1.3	-2.0
4	900	18.4	-2.0	-2.0	-2.9

Table IV *Changes in stroke volume (ml) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	130	-17	-52	-36
2	600	98	-18	-18	- 5
3	600	107	- 7	-24	- 1
4	900	131	+ 6	-28	-19

Table V *Changes in mean pressure (mm Hg) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	97	- 6	-11	-12
2	600	108	- 4	-15	-10
3	600	112	-16	-20	-24
4	900	106	- 1	-12	- 6

Table VI *Changes in peripheral resistance (U) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	9.2	+1.1	+1.6	+1.3
2	600	6.6	+3.9	-0.1	+1.1
3	600	8.0	+0.7	-0.8	-0.7
4	900	5.8	+0.7	-0.1	+0.7

The synergistic effect on angina pectoris found with the combined therapy β blockade and nitroglycerin or other nitrates is probably due at least in part to the prevention of the oxygenconsuming tachycardia caused by nitroglycerin alone as earlier suggested by MacAlpin et al (14) and Russek (17). It might then be expected that the cardiac output would be very much lowered when using these two drugs together but this suspicion could not be verified in the present investigation. Instead the decrease of the cardiac output was of the same order as that caused by only one of the drugs. This was due to the fact that the reduction of the stroke volume produced by nitroglycerin was less pronounced when also propranolol was given. Both drugs are hypotensive but the drop in mean pressure caused by the combination did not seem to be larger than that produced by nitroglycerin alone. The rise in peripheral resistance found after propranolol was however not consistently affected when also nitroglycerin was given.

The combination of propranolol and nitroglycerin thus seems to produce haemodynamic effects which should be favourable for the ischaemic heart i.e. decrease in heart rate, cardiac output and mean pressure. These effects diminish the work of the heart i.e. the oxygen consumption which can then be better adapted to the available oxygen. Other mechanisms may of course also be operative.

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PLASMA HYDROCORTISONE VALUES IN HEART DISEASE

*Results after Acute Myocardial Infarction (with and without Cardiogenic Shock)
and in Patients with Congestive Heart Failure*

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Abstract The plasma hydrocortisone level has been examined in ten patients with acute coronary occlusion and shock in 12 patients with acute coronary occlusion without shock, in seven patients with congestive heart failure and in five control patients. Owing to the circadian rhythm of the plasma hydrocortisone level the examinations have been carried out in five daily determinations on three consecutive days. As compared with the control patients, the average values were found to be significantly higher in patients with congestive heart failure and even higher in the patients with coronary occlusion with and without shock. No correlation was found between the plasma hydrocortisone concentration and the enzymes SGOT and SLDH, the morning temperature or the blood pressure in the patients with acute myocardial infarction. The plasma hydrocortisone level in the patients with coronary occlusion returned to the normal range within 48 hours after admission. The highest values were found in the patients who died and the increased level was maintained until the last determinations before death.

In relation to the discussion about the value of treatment with glucocorticoids in patients with shock following acute coronary occlusion it has seemed natural to establish the spontaneous adrenocortical reaction following a myocardial infarction.

Several investigators have shown that in most cases of acute myocardial infarction the plasma hydrocortisone level is higher during the first couple of days. Logan and Murdoch (7) found that in all their patients the values of plasma hydrocortisone returned to the normal range within three days after the infarction. In addition they found a correlation between the peak plasma hydrocortisone values and the correspond-

ing values of SGOT, which was also found by Bailey et al (1). Logan and Murdoch determined plasma hydrocortisone shortly after admission and thereafter at frequent intervals. Bailey et al took blood samples from most patients at 10 a.m. for 4-6 days. In both these investigations the peak plasma hydrocortisone values were used and no regard was paid to the circadian rhythm of the plasma hydrocortisone level. The above mentioned authors state that the patients had acute myocardial infarction but no information is given about the time elapsing from the onset of the symptoms until admission.

In our investigation the plasma hydrocortisone values have been determined five times daily and all the patients with coronary occlusion were hospitalized within the first 24 hours after the beginning of the symptoms. We have examined a group of patients with acute myocardial infarction with and without shock and we have tried to correlate the plasma hydrocortisone values to the blood pressure and various parameters used in the diagnosis of myocardial infarction. Likewise we have examined the plasma hydrocortisone level in patients with congestive heart failure.

MATERIAL AND METHODS

The investigation comprises

A few of two patients with acute myocardial infarction arisen within the last 4 hours before admission (diagnostic criteria: characteristic precordial pains and electrocardiographic changes, increase of the enzymes SGOT and SLDH and increase of temperature).

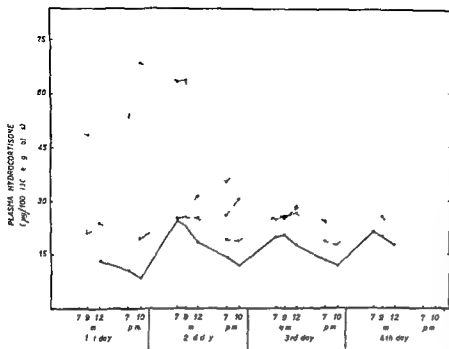


Fig 1 Average values of plasma hydrocortisone in ten patients with acute myocardial infarction and shock (—○—○—) twelve patients with acute myocardial infarction without shock (—○—○—) seven patients with congestive heart failure (—○—○—) and five control patients (—)

Of these patients

1 ten (3 women 5 men aged 52–72) had cardiogenic shock (systolic blood pressure below 90 mm Hg) at some time during the observation period and twelve (12 men aged 41–72) had no cardiogenic shock

B Seven patients with congestive heart failure with out acute myocardial infarction (2 women 5 men aged 39–81)

C Five control patients without pain and without any clinical sign of endocrine disease (2 women 3 men aged 27–53)

Blood samples for determination of plasma hydrocortisone were taken after admission for three days at 7 a.m. 9 a.m. 12 a.m. 7 p.m. and 10 p.m. which meant 15 samples from surviving patients. At the same time the blood pressure was measured. The patients with shock were treated with intravenous infusion of metaradren

The blood samples for determination of SGOT and SLDH values in the patients with myocardial infarction were taken in the morning and we tried to correlate these values to the plasma hydrocortisone concentration at 9 a.m. and to the morning temperature and the blood pressure at 9 a.m.

The plasma hydrocortisone estimations were carried

Table I Mean values of plasma hydrocortisone in five controls (see text)

	7 a.m.	9 a.m.	12 a.m.	7 p.m.	10 p.m.
No of observations	11	15	15	15	15
Mean value (µg/100 ml)	22.1	21.3	17.0	12.9	10.9
s.d.	5.4	4.6	5.0	3.1	4.3

Table II Mean values of plasma hydrocortisone in seven patients with congestive heart failure

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
	12 a.m.	7 p.m.	10 p.m.	7 a.m.	9 a.m.	12 a.m.	7 p.m.	10 p.m.
No of observations	2	7	7	6	7	7	7	6
Mean value (µg/100 ml)	24.0	21.0	19.7	25.0	25.5	25.3	20.9	18.7
s.d.	0.0	5.6	5.0	4.0	6.6	5.3	9.2	9.3
Significance	$p < 0.001$	$p < 0.01$	$p < 0.05$	n.s.	n.s.	$p < 0.005$	n.s.	n.s.

n.s. — not significant

out by Medicinsk Laboratorium Copenhagen using the fluorimetric method of De Moor et al. (3, 4)

The statistical calculations were done with a computer (IBM 1130)

RESULTS

The average values of plasma hydrocortisone in the different groups of patients are shown in Fig 1

It seems from the curve comprising the controls that the values at the same hour are the same from day to day and by analysis of variance it is confirmed that there is no significant difference. We have therefore used the values from all three days when calculating the average values of plasma hydrocortisone in the control patients at each individual hour and the results appear from Table I. On the other hand the analysis of variance shows a significant difference between the values at the different moments within the same 24 hours ($F=4.444$ $p<0.01$)

After admission the patients with congestive heart failure have increased plasma hydrocortisone values and even higher values are found in the patients with acute myocardial infarction without shock. The highest values are seen in the patients with acute myocardial infarction and cardiogenic shock. This appears from Fig 1 and the average values are shown in Tables II, III and IV together with the results of the statistical analysis. Here the mean plasma hydrocortisone values of the different groups of patients are appraised in relation to the mean values of the control group.

The highest plasma hydrocortisone value we have recorded was $164 \mu\text{g ppr } 100 \text{ ml}$. The highest values were found in the patients who died and the increase persisted until the last determinations before death. In the group with myocardial in-

farction without shock one patient died (23 days after admission) in the group with myocardial infarction and cardiogenic shock six patients died (from 20 hours to 13 days after admission)

Table V shows the last values of plasma hydrocortisone recorded in the patients who died

Even in the patients with acute myocardial infarction and shock the average values of plasma hydrocortisone return to the normal range within 48 hours. Until then the values are significantly higher. The range is great in the period when the values are increased but during the first 48 hours all patients with acute myocardial infarction and shock still have values above the mean for controls. In the group with acute myocardial infarction without shock we have recorded during the first 24 hours one plasma hydrocortisone value within the normal range while during the next 24 hours we found 9 out of 57 observations to be within the normal range.

In the patients with acute myocardial infarction with and without shock, we have tried to correlate the plasma hydrocortisone values at 9 a.m. to the morning temperature, to SGOT, to SLDH and to the systolic and diastolic blood pressure. The results appear from Tables VI and VII. We have found no significant correlation in the patients with coronary occlusion without shock. In the patients with acute coronary occlusion and shock we have found a correlation ($p<0.05$) between plasma hydrocortisone and the diastolic blood pressure at 9 a.m. We also found a significant correlation between plasma hydrocortisone and SLDH but after exclusion of a single extremely high SLDH value there was no significance.

DISCUSSION

The examinations confirm earlier observations that the plasma hydrocortisone level is elevated in patients with acute myocardial infarction. The increase is higher when cardiogenic shock occurs. The examination of our control patients shows that admission per se does not influence the plasma hydrocortisone concentration.

It is still discussed what causes the increased adrenocortical activity in patients with acute coronary occlusion. Logan and Murdoch (7) as well as Bailey et al. (1) suppose that the actual myocardial necrosis is the essential factor. This

hrd					
a.m.	9 a.m.	12 a.m.	7 p.m.	10 p.m.	
7	6	6	6	6	
89	258	270	188	178	
31	73	26	62	63	
s.	n.s.	$p<0.01$	n.s.	n.s.	

Table III Mean values of plasma hydrocortisone in twelve patients with acute myocardial infarction without shock

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
	12 a.m.	7 p.m.	10 p.m.	7 a.m.	9 a.m.	12 a.m.	7 p.m.	10 p.m.
No. of observations	6	9	12	12	11	10	12	12
Mean value ($\mu\text{g}/100\text{ ml}$)	39.0	35.0	28.0	34.1	23.6	31.3	26.2	30.7
S.D.	9.9	21.5	17.8	18.2	12.7	11.2	1.3	14.1
Significance	$p < 0.01$	n.s.	$p < 0.01$	n.s.	n.s.	$p < 0.05$	$p < 0.005$	$p < 0.001$

Table IV Mean values of plasma hydrocortisone in ten patients with acute myocardial infarction and shock

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
	12 a.m.	7 p.m.	10 p.m.	7 a.m.	9 a.m.	12 a.m.	7 p.m.	10 p.m.
No. of observations	9	10	9	9	8	8	8	7
Mean value ($\mu\text{g}/100\text{ ml}$)	55.0	53.4	57.4	53.0	58.9	40.4	35.8	39.1
S.D.	23.0	29.5	25.4	30.5	28.3	18.3	19.2	18.7
Significance	$p < 0.001$	$p < 0.01$	$p < 0.001$	n.s.	$p < 0.01$	$p < 0.01$	n.s.	$p < 0.01$

Table V The last recorded plasma hydrocortisone values in patients who died

Diagnosis	Plasma hydrocortisone ($\mu\text{g}/100\text{ ml}$)	Time after admission for	
		Plasma hydrocortisone	Death
Acute myocardial infarction + shock	4.	7 a.m. 4th day	7 d
Acute myocardial infarction + shock	130	10 p.m. 1st day	(9 h before death) 0 h
Acute myocardial infarction + shock	74	7 p.m. 3rd day	(2 h before death) 44 h
Acute myocardial infarction + shock	105	9 a.m. 2nd day	(1 h before death) 4 h
Acute myocardial infarction + shock	37	7 p.m. 3rd day	13 d
Acute myocardial infarction + shock	30	9 a.m. 4th day	5 d
Acute myocardial infarction without shock	38	10 p.m. 3rd day	3 d
Congestive heart failure	4	7 a.m. 3rd day	7 d

Table VI Twelve patients with acute myocardial infarction without shock

Plasma hydrocortisone correlated to blood pressure, rectal temperature, SGOT and SLDH

Variable Y	Variable X	No. of observations (n)	Correlation coefficient (r)	Probability (p)
Plasma hydrocortisone at 9 a.m.	Systolic BP at 9 a.m.	35	-0.0169	n.s.
Plasma hydrocortisone at 9 a.m.	Diastolic BP at 9 a.m.	35	-0.1802	n.s.
Plasma hydrocortisone at 9 a.m.	Morning temperature	32	-0.0505	n.s.
Plasma hydrocortisone at 9 a.m.	SGOT	35	0.1433	n.s.
Plasma hydrocortisone at 9 a.m.	SLDH	34	0.056	n.s.

Third

Time	9 a.m.	12 a.m.	7 p.m.	10 p.m.
11	9	12	12	10
50	25.9	28.7	25.8	22.1
91	10.1	9.0	10.7	11.2
15	n.s.	$p < 0.001$	$p < 0.01$	n.s.

Fourth

Time	9 a.m.	12 a.m.	7 p.m.	10 p.m.
7	7	7	7	6
97	25.3	28.9	24.4	20.0
71	9.5	9.6	9.1	9.2
15	n.s.	n.s.	n.s.	n.s.

is supported by the fact that these investigators did not find any increased level of plasma hydrocortisone in patients with precordial pain without myocardial infarction. In the same materials there was a correlation between the peak plasma hydrocortisone values and the peak SGOT values.

Oka (8) however who determined the free 17 hydroxycorticosteroids in plasma also found increased values in patients with stenocardia without any sign of myocardial infarction.

We have not been able to demonstrate a cor

relation between the plasma hydrocortisone level and the SGOT concentration. We have chosen to compare values taken at the same time instead of using the peak values. This is also the case with regard to SLDH and furthermore as the examination period was three days we have in several cases most likely not recorded the peak SLDH value at all as it might appear later. The correlation we have found between the plasma hydrocortisone level and the diastolic blood pressure in patients with acute myocardial infarction and shock is hardly real. All the patients with shock were treated with metaradrin at different times during the observation period. The blood pressure figures are therefore influenced by the treatment and do not indicate the spontaneous course.

We have thus been unable to show any significant correlation between the plasma hydrocortisone level and the other parameters used in our examination of patients with acute coronary occlusion.

We have found the highest plasma hydrocortisone values in the patients who died. This might support the presumption made by Klein and Palmer (5) that the prognosis is worse if the initial plasma hydrocortisone concentration exceeds 40 μ g per 100 ml. However several of our patients survived with higher values.

In the material of Bailey et al. (1) there was one patient who died 11 hours after admission with a normal level of plasma hydrocortisone immediately before death. In our investigation all the patients who died had increased values at the last determinations.

If the myocardial necrosis causes the increase of plasma hydrocortisone the question arises why

Table VII Ten patients with acute myocardial infarction and shock.

Plasma hydrocortisone correlated to blood pressure, rectal temperature, SGOT and SLDH

Variable Y	Variable X	No. of observations (n)	Correlation coefficient (r)	Probability (p)
Plasma hydrocortisone at 9 a.m.	Systolic BP at 9 a.m.	24	0.3943	n.s.
Plasma hydrocortisone at 9 a.m.	Diastolic BP at 9 a.m.	11	0.52.6	$p < 0.05$
Plasma hydrocortisone at 9 a.m.	Morning temperature	23	0.3197	n.s.
Plasma hydrocortisone at 9 a.m.	SGOT	22	0.4162	n.s.
Plasma hydrocortisone at 9 a.m.	SLDH	22	0.5938	$p < 0.01$
Plasma hydrocortisone at 9 a.m. after deduction of 1 patient with extremely high SLDH value	SLDH	21	0.1348	n.s.

the patients with congestive heart failure also have increased values. Among our patients in this group the average values of plasma hydrocortisone are significantly higher during the first 24 hours after admission. These patients had most likely no myocardial necrosis.

Knapp et al (6) examined the circadian rhythm of plasma 11 hydroxycorticosteroids in 10 patients with congestive heart failure. The average values were determined on the basis of seven observations in 24 hours. The curve did not show the characteristic variation. The values were within the normal range from 8 a.m. to 4 p.m. After that time the level was above normal. Contrary to the above mentioned examination several values in our patients were increased also about noon.

Connolly and Wills (2) examined plasma cortisol at midnight and at 9 a.m. after the first day in hospital in six patients with congestive heart failure (one patient with myocardial infarction). All the patients had increased values at midnight and three (including the patient with myocardial infarction) had also values above normal at 9 a.m. The authors draw attention to the fact that with the technique used by them both cortisol and corticosterone were determined. This is also the case in our investigation. Congestive heart failure can be followed by secondary hyperaldosteronism and therefore the estimated high level may be due to an increased corticosterone concentration.

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THE VALUE OF PBI AND T_3 TEST IN AN ENDEMIC GOITER AREA

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Abstract An investigation of the value of the PBI and of the tri-iodothyronine (T_3) test in an endemic goiter area shows that in euthyroid and hypothyroid cases PBI gives pathologically raised values in 30%. The T_3 test in the same material gave incorrect values the figure usually being too low in 8% of the euthyroid cases. In the whole material (340 patients) PBI gave incorrect information in 28% the T_3 test in 9% and free thyroxine index* ($PBI \times T_3$) in 7%. In an endemic goiter area the diagnosis of the functional condition of the thyroid is considerably more difficult since tracer iodine examination as well as PBI determination often give values expressing hyperthyroidism in euthyroid cases. Clinical assessment of the patients is therefore of specially great value in an endemic goiter area.

In an endemic goiter area our usual laboratory methods in thyroid diseases not seldom give incorrect results. In iodine tracer examinations one fairly often gets hyperthyroid values i.e. the I^{131} taken up by the thyroid gland is increased and that which is excreted in the urine is reduced in spite of the patient being euthyroid. In a work published earlier we have shown that this occurred in 51 out of 256 patients (7). PBI I^{131} determination in these cases has proved to be a valuable complement (10) but here too one can get raised values in euthyroid patients as in cases of small active adenomata or after treatment with radio-active iodine.

Raised PBI values are not a rare occurrence in euthyroid patients with endemic goiter (6, 13). The cause of the raised PBI values would seem to be that the protracted lack of iodine has damaged the thyroid gland so that biologically inactive or low active iodine compounds are released into the blood and are included in the determination. Mono- and di-iodotyrosine together with thyroglobulin do not normally occur in the blood but may appear in the event of injury to or disease of the thyroid.

If mono-iodotyrosine is added in vitro one may find in PBI about 20% of the added activity while of added di-iodotyrosine 50% may be found in PBI. Consequently the PBI value is influenced considerably by the low active di-iodo- and mono-iodotyrosine pole (8).

The determination of BEI is in this respect much more accurate since mono- and di-iodotyrosine practically speaking are not included in this method at all. Determination of the BEI value is however a far too complicated method for routine use.

Dowling et al. found a raised PBI value in five euthyroid adults with goiter and some of them exhibited a nonbutanol-extracted iodised peptide or protein of thyroid origin. These patients also had a high uptake of I^{131} (5).

The adsorption of radio-active tri-iodothyronine to Sephadex after being added to serum (T_3 test) is used generally as a thyroid function test and lacks a number of the disadvantages of PBI e.g. sensitivity to iodine contamination. The T_3 test reflects the degree of unsaturated thyroxine binding protein (TBP). This test also has its limitations due chiefly to changes in TBP which are not caused by the thyroid. When TBP increases so also does PBI to a certain degree while the T_3 value is reduced (e.g. pregnancy, contraceptive pills) (4, 9). On reduction of TBP (e.g. nephrosis) PBI is reduced while the T_3 value is raised (14).

Clark and Horn (3) suggested a so-called free thyroxine index calculated as the product of the values for PBI and T_3 . This is an arbitrary figure which should be proportional to free thyroxine in the blood.

Nossin (12) found correct values for PBI in 84% for the T_3 test in 85% and for free thyroxine index in 91%. The material which is not from an endemic goiter area comprised 23 hypothyroid

the product of $PBI \times T_3$ in the various

(June 4-10)

	Total no	Too high	Too low
total	284	70	1
euthyroid	73	—	—
hypothyroid	33	—	1

Table IV The whole material

	Right	Wrong	Error (%)
PBI	245	95	28
T_3	308	32	9
$PBI \times T_3$	316	44	7

DISCUSSION

In an endemic goiter area PBI is a bad standard of value regarding the patient's thyroid status especially in differentiating between hyper or euthyroidism. In our material PBI was raised in 30% of euthyroid patients without any iodine contamination or treatment being traceable which could effect the determination. In seven cases of 23 (30%) of hypothyroidism PBI was above the lower normal limit.

The T_3 test seems to us to be a valuable complement as in the whole material this test was only wrong in 9%. Among the euthyroid patients (284 in total) there were five above and 19 below the normal limits which implies an error of 8%. Also with hypothyroidism the T_3 test seems to be more reliable as in our material only 9% of the T_3 values were raised while the corresponding figure for PBI here was 30%.

The product of PBI and T_3 (free thyroxine index) in this investigation does not give much better values than the T_3 test. This is due to two reasons. The first is the high percentage of raised PBI values in our material whereby the product also becomes raised. The second is that as far as possible we excluded from the material those persons concerning whom there was reason to suspect disturbances in TBP. In these cases on the contrary the product of the PBI and T_3 test gave considerably better results than the PBI and T_3 values alone since disturbances in TBP change the values of PBI and T_3 in the opposite direction.

Our investigations of the reliability of the laboratory methods in an endemic goiter area have shown that PBI and tracer iodine estimations give incorrect values in the hyperthyroid reaction. The determination of PBI¹³¹ and the radiothyronine test give considerably less accurate values. In spite of all new laboratory methods which have come into being for the function of the thyroid the clinical assessment has its great value and is a necessary important factor in the determination of the function.

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Table I The PBI values for the various groups

PBI (normal value 4-8)

	Total no	Too high	Too low
Euthyroid	284	86 (30 %)	0
Hypothyroid	23	7 (30 %)	—
Hyperthyroid	33	—	2

77 euthyroid and 41 hyperthyroid cases. He considers that the index seems to be of special value in cases where both PBI and T_3 test are disturbed because of an abnormal TBP concentration such as is found in pregnancy, women taking contraceptive pills, nephrosis, cirrhosis etc.

Also Andersson (1) considers that the product of $PBI \times T_3$ forms a very useful indirect determination of free thyroxin and thereby of the patient's thyroid status. This idea is also shared by Wellby and O'Halloran (16).

As we have not infrequently obtained PBI values deviating from the clinical picture we have wished to correlate these to the value of the T_3 test and free thyroxin index (the product of $PBI \times T_3$).

MATERIAL

All patients upon whom PBI and T_3 tests had been made during the period February 1965 to June 1966 inclusive

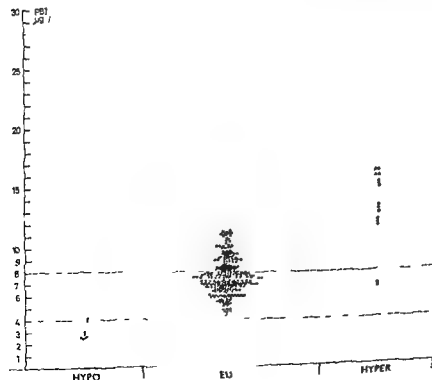


Fig. 1

have been clinically judged by one or more doctors in the Medical Clinic, Sundsvall Hospital, which is situated in an endemic goiter area. All patients with suspected iodine contamination and those who had been treated with oestrogens (e.g. contraceptive pills), androgens and thyroid substitute have been excluded from the material. Those with seriously changed serum proteins and thereby presumed TBP displacements as well as patients with cirrhosis of the liver, nephrosis and malignancy have not been included. Pregnant patients have likewise been excluded. No operated or I^{131} treated case is included except in the hypothyroid material, since otherwise this latter group would have been very small. Only patients for whom a definite clinical diagnosis could be made with regard to the thyroid function have been included.

After this thinning-out, the material consisted of 340 patients, of whom 284 had been diagnosed as euthyroid, 23 as hypothyroid and 33 as hyperthyroid. The clinical diagnosis was confirmed in the two latter groups by cholesterol in the serum, iodine tracer examinations, FCG and the result of specific treatment. In cases where we found very high PBI values and iodine supplies could not be anamnestically excluded, iodine-tracer examinations have been carried out. Patients with signs of blocking have been assumed to be iodine-contaminated and excluded from the material.

METHODS

PBI has been determined by a method Skäns and Hedenskog (15) which is a modification of a method worked out by Barker (?). The for healthy individuals is 59 μ g per l.

standard deviation of 0.7 μg per 100 ml for both sexes. The normal limits are 3.8–8 μg per 100 ml. In practice the limits have been set at 4–8 μg per 100 ml.

Triiodothyronine determination (T_3 test) was carried out by a method described by Nossin (11) in which Sephadex was used to adsorb triiodothyronine which is not bound to serum protein. The result is expressed in percent and the normal value is 80–120%. Lower values are seen in cases of hypothyroidism or raised TBP and higher values in cases of hyperthyroidism or lowered TBP.

Free thyroxin index according to Clark and Horn (3) has been calculated as the mathematical product of PBI and T_3 divided by 100.

RESULTS

The PBI values for the various groups are shown in Table I and Fig. 1.

In both the euthyroid and the hypothyroid groups not less than 30% had too high values while in no case was PBI incorrectly low. In 6% of the hyperthyroid cases too low PBI values were found.

Table II and Fig. 2 show the values for the T_3 test.

The T_3 test gave incorrect values in 8% of the euthyroid cases and in the majority of these cases the value was too low. In the hypothyroid cases

Table II The T_3 test values for the various groups
 T_3 test (normal value 80–120%)

	Total no	Too high	Too low
Euthyroid	24	5	19
Hypothyroid	23	2	—
Hyperthyroid	33	—	6

the value was too high in 9% and in the hyperthyroid patients too low in 18%.

Table III shows the product of $\text{PBI} \times T_3$ in the various groups.

The calculated percentage error for free thyroxin consequently means that 7% of the euthyroid patients had a value which was wrong being almost exclusively too high. Furthermore a too high product value was found in 9% of the hypothyroid cases and too low a value in 3% of the thyrotoxic patients.

Table IV shows the number of inaccurate values in the whole material partly in actual figures and partly in percentages.

Consequently PBI gave an inaccurate value in 28% of the material, the T_3 test in 9% and the free thyroxin index in 7%.

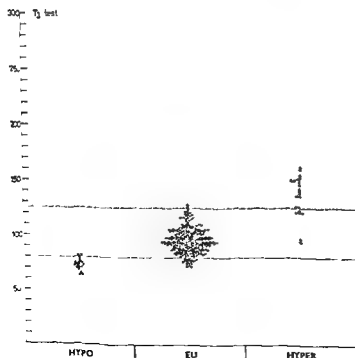


Fig. T_3 test values for the various groups.

Table III The product of $PBI \times T_3$ in the various groups $PBI \times T_3$ (normal value 4-10)

	Total no	Too high	Too low
Euthyroid	284	20	1
Hypothyroid	23	2	—
Hyperthyroid	33	—	1

Table IV The whole material

	Right	Wrong	Error (%)
PBI	245	95	28
T_3	308	32	9
$PBI \times T_3$	316	4	7

DISCUSSION

In an endemic goiter area PBI is a bad standard of value regarding the patient's thyroid status especially in differentiating between hyper or euthyroidism. In our material PBI was raised in 30% of euthyroid patients without any iodine contamination or treatment being traceable which would effect the determination. In seven cases of (30%) of hypothyroidism PBI was above the lower normal limit.

The T_3 test seems to us to be a valuable complement, as in the whole material this test was only wrong in 9%. Among the euthyroid patients (284 in total) there were five above and 19 below the normal limits which implies an error of 8%. Also with hypothyroidism the T_3 test seems to be more reliable as in our material only 9% of the T_3 values were raised while the corresponding figure for PBI here was 30%.

The product of PBI and T_3 ("free thyroxine index") in this investigation does not give much better values than the T_3 test. This is due to two reasons. The first is the high percentage of raised PBI values in our material whereby the product also becomes raised. The second is that, as far as possible, we excluded from the material those persons concerning whom there was reason to suspect disturbances in TBP. In these cases on the contrary the product of the PBI and T_3 test gave considerably better results than the PBI and T_3 values alone since disturbances in TBP change the values of PBI and T_3 in the opposite direction.

Our investigations of the reliability of different laboratory methods in an endemic goiter area have shown that PBI and tracer iodine examinations give incorrect values in the hyperthyroid direction. The determination of PBI¹²¹ and the triiodothyronine test give considerably less inaccurate values. In spite of all new laboratory investigations which have come into being for judging the function of the thyroid the clinical assessment still has its great value and is a necessary and important factor in the determination of the thyroid function.

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UREMIC PERICARDITIS

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Abstract The frequency of uremic pericarditis and complicating cardiac tamponade has been determined in a series of 175 patients in terminal uremia. Forty (32%) were found to have uremic pericarditis and five of these patients (12.5%) developed a pericardial effusion sufficient to produce cardiac tamponade. The diagnosis of uremic pericarditis had been made during life in 32 of the 40 patients on the presence of a pericardial friction rub which was found to be the most constant symptom of pericarditis. Demonstration of acute pericardial constriction was solely clinical rapidly developing cardiac insufficiency in a clinical situation where hypertensive heart disease overhydration and severe anemia could be ruled out as etiologic factors.

Seven of the patients with uremic pericarditis were treated with regular hemodialysis using universal heparinization. One patient developed cardiac tamponade in connection with his first hemodialysis. All the rest of the hemodialyses in this patient and in the other patients were without complications. The risk of universal heparinization must therefore be considered to be minimal. The size and shape of the heart became normal in three of the seven patients and the pericardial friction rub disappeared in all of them during intermittent hemodialysis. It is thus possible with effective hemodialysis to treat uremic pericarditis and complicating pericardial effusion so that renal allotransplantation can be carried out. Eight patients with uremic pericarditis in this series including two with cardiac tamponade have received renal transplants. Seven of these patients are alive one three years post transplant.

Pericarditis commonly accompanies terminal uremia. The presence of this complication was previously considered to mean that death was impending (12). Intermittent dialysis and renal transplantation have, however, changed the prognosis of terminal uremia and of the complications that accompany it so that uremic pericarditis can now be considered accessible to treatment (1, 2, 3, 4, 5, 6, 7, 11).

In the present work a series of uremic patients is analysed in order to determine the frequency and clinical manifestations of uremic pericarditis

as well as to elucidate the treatment of this condition.

MATERIAL AND METHODS

In the period April 1964 to October 1968 125 patients with terminal uremia i.e. $Cl_0 < 5$ ml/min were admitted to this department. There were 62 men and 63 women with ages ranging from 16 to 87 years. The etiology of the renal diseases involved is given in Table I. Seventy-five of these patients were treated with intermittent peritoneal dialysis, 36 with intermittent hemodialysis and in 65 renal allotransplantation was carried out.

Pericarditis was diagnosed in 40 of these 125 uremic patients (32%). 33 of the 40 died and autopsy was performed on 32. General data concerning these 40 patients is given in Table II. In 32 patients the diagnosis of uremic pericarditis was made in vivo on the presence of a pericardial friction rub; in the other eight the diagnosis was based on autopsy findings. There was no apparent difference between the 40 uremic patients who developed pericarditis and the 85 who did not; both groups were of approximately the same age and sex distribution, the renal diseases involved were not different and the severity of uremia was the same in both groups.

Five of the patients with uremic pericarditis developed an acute pericardial constriction syndrome (12.5%).

RESULTS

Pericarditis

A pericardial friction rub was heard in 32 out of 40 patients (80%). The friction rub varied in strength from barely audible to loud enough to be heard with the stethoscope above the chest wall and to be accompanied by a palpable thrill. Pain was not a pronounced symptom as only 14 of the 40 patients complained of it. When felt pain was localized to the precordium and was often described as oppressive. Only one patient complained of intense precordial pain with radiation to the back and left shoulder. In almost all the patients pain increased on deep breathing.

Table I 125 patients in terminal uremia etiology

Chronic glomerulonephritis	45
Acute glomerulonephritis	3
Chronic pyelonephritis	51
Medullary cystic disease	5
Renal hypoplasia	5
Hereditary nephritis	4
Nephrosclerosis	3
Obstructive nephropathy	2
Renal amyloidosis	2
Diabetic nephropathy	2
Polycystic kidneys	1
Renal sarcoidosis	1
Radiation nephritis	1

Table II Uremic pericarditis general data

Total patients	125
No. with pericarditis	40
Males	19
Females	21
Age range (y)	16-68
Incidence (%)	32
Renal disease	
Chronic glomerulonephritis	19
Chronic pyelonephritis	14
Medullary cystic disease	2
Polycystic kidneys	1
Renal hypoplasia	1
Diabetic nephropathy	1
Renal amyloidosis	1
Renal sarcoidosis	1

Electrocardiograms were taken frequently in all patients and abnormalities were seen in 18 (45%) (Table III). Characteristic signs of pericarditis in the form of S-T segment elevation were seen in ten patients. These changes were followed in the course of three to 14 days by depression and inversion of T waves in six patients. Low voltage appeared in four patients, three of which proved to have a pericardial effusion. A normal electrocardiogram—excepting the changes secondary to arterial hypertension—was seen in 22 patients. In six patients disturbances in cardiac rhythm: auricular flutter, auricular fibrillation or nodal rhythm were seen. Two patients had in addition right axis deviation and one had a right bundle branch block.

Thirty-eight patients (95%) evidenced cardiac enlargement of varying degree (Table III). In seven of these patients X-ray films suggested a pericardial effusion, i.e. there was a flattening out of the cardiac contour. The characteristic

tent shaped cardiac silhouette was seen in only three cases.

Earlier the most important consequence of the diagnosis of uremic pericarditis was that it indicated the fatal outcome of uremia in the course of a few weeks. Survival before the advent of dialysis was among our patients two to 30 days. In three patients however we have seen transitory pericarditis in association with dehydration, acidosis, infection and papillary necrosis. After correction or disappearance of these conditions the symptoms of pericarditis were no longer present. One of these patients died a year later because of uremia. The other two did not develop uremic pericarditis during the following 2½ years in spite of progressive renal failure.

Cardiac tamponade

This was seen in five patients. Azotemia was most marked among this group: creatinine clearance was <2 ml/min, the mean value for plasma creatinine was 210 mg/100 ml and for blood urea 410 mg/100 ml (Table IV). Pericarditis had been present in all of these patients for some length of time, how long cannot be stated as many patients had pericarditis on admission.

Table III Clinical features of uremic pericarditis

Presenting feature	
Friction rub only	18
Friction rub and chest pain	14
ECG abnormalities total no.	18
S-T elevation	10
Inversion of T waves	6
Low voltage	4
Arrhythmia: atrial flutter	5
nodal rhythm	1
Right axis	2
Normal electrocardiogram	2
X-ray	
Cardio-thoracic ratio ≤ 0.50	2
Cardio-thoracic ratio > 0.50 - < 0.60	2
Cardio-thoracic ratio > 0.60 - < 0.70	13
Cardio-thoracic ratio > 0.70	1
Suggestion of pericardial effusion	7
Pain	
Absent	17
Present	13
With radiation	1
Cardiac tamponade	
No of pericardiocenteses	3
Death resulting from tamponade	3

Left ventricular enlargement from long standing hypertension was disregarded.

Table IV Comparison of mean laboratory values at onset of pericarditis with those at time of clinical disappearance in patients in terminal uremia

	Onset		Disappearance	
	Plasma-creatinine (mg/100 ml)	Blood-urea (mg/100 ml)	Plasma-creatinine (mg/100 ml)	Blood-urea (mg/100 ml)
Group I (17 patients) Pericarditis persisting until death	14.0	325		
Group II (23 patients) Pericarditis treated with dialysis	20.5	340	10.5	152
Group III (5 patients) Pericarditis associated with cardiac tamponade	21.0	410	15.0	250

There was roentgenographic evidence of increasing cardiac size during the days immediately preceding the development of the acute constriction syndrome. In two cases the electrocardiogram showed low voltage and in two other patients there was S-T segment elevation and inverted T waves. Two patients had auricular fibrillation. The clinical symptoms of cardiac tamponade developed in these patients during the course of a few hours and were characterized by signs of right heart failure with dyspnea, marked engorgement of neck veins and an enlarged liver. The patients were lethargic, gray-sallow in color and had a falling blood pressure and pulse pressure and increasing peripheral venous pressure. The friction rub decreased or disappeared in connection with the appearance of the pericardial effusion.

One patient developed an acute constriction syndrome during his first hemodialysis with universal heparinization (Table V case 1). A few days after pericardiocentesis, intermittent hemodialysis with universal heparinization was again started. Sixteen complication-free dialyses were performed. Azotemia was brought under control and the symptoms of uremic pericarditis disappeared. In another patient (Table V case 6 and Fig. 1) cardiac tamponade appeared during insufficient intermittent peritoneal dialysis. On intensive hemodialysis treatment the uremic pericarditis disappeared and the heart size became normal.

The acute constriction syndrome was rec-

ognized *in vivo* in four patients but in one patient the diagnosis was first made on autopsy. Pericardiocentesis was done six times with the removal of from 225 to 1360 ml of serosanguinous fluid. This procedure had prompt clinical and circulatory effect. No complications were observed in connection with pericardiocentesis.

Recovery from pericardial effusion and tamponade

Two characteristic cases of uremic pericarditis with accompanying cardiac tamponade are reviewed below.

Case 1

A 29-year-old man was discovered to have renal disease in 1960 at the age of 21. The patient was clinically well until July 1966 when advanced renal insufficiency was demonstrated. On admission to our department on 5 August 1966 the plasma creatinine was 17.5 mg/100 ml and there was severe hypertension with grade IV hypertensive retinopathy and retinal detachment together with overhydration.

The azotemia was controlled by intermittent peritoneal dialysis and the blood pressure reduced to 150/160/105-115 mm Hg on treatment with antihypertensive agents. There was no clinical or roentgenologic evidence of pericarditis. X-ray of the chest 21 days after admission revealed a slightly increased cardiac diameter (cardio-thoracic ratio 17.3/33.0 cm).

Forty days after admission the blood pressure began to fall and in the course of the following six days dropped from 155/110 to 100/90 and the pulse increased from 80 to 120 per min. A chest X-ray 45 days after admission showed a tent-shaped cardiac silhouette and there was S-T segment elevation but normal voltage

Table V Patients with pericarditis and roentgenological evidence of pericardial effusion treated with hemodialysis and universal heparinization

At start of hemodialysis			Pretransplantation			Outcome
Case no	Average creatinine clearance (ml/min)	Chest film suggestive of pericardial effusion	No of dialyses	Cardiac tamponade during hemodialysis	Chest film showing cardiac dilatation	
1	1	+	17	+	+	Transpl 18 11 1966 Normal renal function Creatinine clearance 70 ml/min
2	0-1	+	14	-	+	Transpl 7 11 66 Normal renal function Creatinine clearance 65 ml/min
3	0-1	-	7	-	-	Transpl 24 1 68 Normal renal function Creatinine clearance 100 ml/min
4	1-2	-	2	-	+	Transpl 28 2 68 Normal renal function Creatinine clearance 60 ml/min
5	3-4	-	51	-	+	Second transpl 20 10 68 Creatinine clearance 30 ml/min
6	0	+	27	-	-	Transpl 28 8 68 Normal renal function Creatinine clearance 70 ml/min
7	1-2	-	14	-	-	Transpl 8 3 67 Initially normal renal function Since August 1967 chronic rejection Died 1 2 68

n the electrocardiogram. The plasma creatinine was 1.5 mg/100 ml at this time.

The following day the patient felt short of breath. At the start of hemodialysis no 1 with universal heparinization, the patient collapsed; the blood pressure was 70/40 and the pulse weak—approx. 90/min. The patient complained of pain in the left side of the chest. He was listless and his face had a pale gray color. Respiration was weak and irregular. The heart sounds were indistinct; the apical beat was felt at the anterior axillary line. No pericardial friction rub was heard. The clinical condition remained unchanged during the following hour and therefore pericardiocentesis was performed with the removal of 1360 ml of sero-sanguinous fluid. There was a prompt clinical and circulatory effect: the blood pressure rose to 140/80 and the pulse became strong and regular—approx. 100 per min. Roentgenographically no change in the cardiac form or size could be demonstrated after pericardiocentesis.

The patient's general condition improved during the course of the following months as a result of intermittent hemodialysis. Renal allotransplantation was performed 103 days after admission and now two years later the patient has normal graft function (Cl_C 70 ml/min) and the blood pressure is normal. The retinal lesions have healed and the heart has a normal form and size on X-ray. The patient has returned to work and is fully employed.

Comment

After precursory roentgenological and electrocardiographical evidence of increasing pericardial effusion this patient developed an acute pericardial constriction syndrome during his first hemodialysis with universal heparinization. After pericardiocentesis 16 additional hemodialyses with universal heparinization were performed without complications. The heart decreased in size during dialysis treatment but normal cardiac form and size was first obtained after renal allotransplantation.

Case 6

A 30-year-old man, who at the age of 5 developed acute glomerulonephritis after an attack of scarlatina.

On 6 May 1968 the patient was admitted in terminal uremia with a plasma creatinine of 30.5 mg/100 ml. He was severely overhydrated. Blood pressure was 200/120 mm Hg and the eyegrounds revealed grade IV hypertensive changes. The patient was oliguric on admission and, from the 4th post admission day anuric. Intermittent dialysis was started immediately and after the first three peritoneal dialyses the patient had sustained a weight loss of 7 kg, but marked azotemia was still present (Fig. 1).

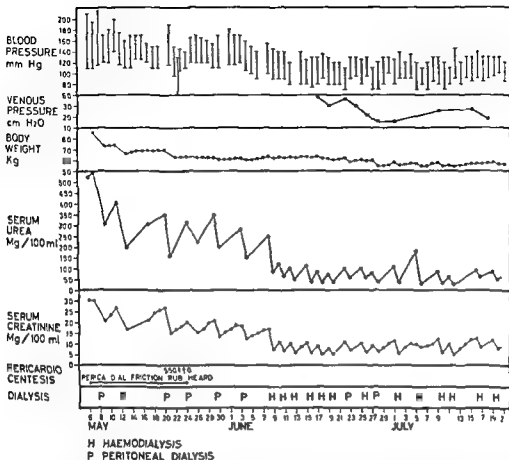


Fig 1 Case 6 The clinical course in a 30 year-old man who on admission on 6 May 1968 was severely overhydrated and markedly uremic. Pericardiocentesis was performed on May 2, and 23 because of cardiac tamponade which developed as a complication of uremic pericarditis. During intensive chronic dialysis azotemia

was controlled and the signs of pericardial constriction lessened. Bilateral nephrectomy was carried out on 5 July 1968 and renal allotransplantation 23 days later. Four weeks after transplantation the creatinine clearance was 60 ml/min, the blood pressure normal and the size and shape of the heart were within normal limits.

A pericardial friction rub was present on admission and persisted despite dialysis. Increasing neck vein engorgement and progressive cardiac enlargement were noted. On the evening of the 15th day in hospital the patient complained of oppressive chest pain and dyspnea. His condition worsened rapidly in the course of the next few hours. Blood pressure fell from 160/100 to 125/60, the apical beat could not be felt, the pericardial friction rub decreased in strength, the neck veins were engorged and the liver margin was felt 5-6 cm below the right costal margin. The patient was ashen in color and severely dyspnoeic. On pericardiocentesis 540 ml sero-sanguinous fluid was removed. His condition improved somewhat thereafter. The following day an X-ray film of the chest showed increasing cardiac enlargement and again there were clinical signs of right heart failure. Pericardiocentesis was again performed, but this time it was not possible to aspirate fluid.

The clinical condition remained critical but on in-

tensification of dialysis it was possible to eliminate the overhydration and to keep the azotemia at a sufficiently low level (Fig 1). The venous pressure fell, liver size decreased and the cardiac silhouette became normal (Fig 2b). The patient was treated with intermittent hemodialysis for 3 months. Universal heparinization was used during all dialyses without complication. On 5 August 1968 bilateral nephrectomy was performed and 23 days later the patient received a donor kidney from his mother. Graft function is now two months later good with a creatinine clearance of 60 ml/min. Blood pressure is normal and X-ray of the chest shows a heart of normal size and shape.

Comment

This patient was severely uremic and overhydrated on admission.



Fig 7 (a) Case 6 X-ray of the chest taken in the supine position on 2 May 1968 before the removal of 540 ml sero-sanguinous fluid from the pericardium. There is marked cardiac enlargement. An invagination is still seen between the aortic arch and the right atrium and thus

the characteristic tent-shaped cardiac silhouette is not present (b) Case 6 X-ray of the chest taken in the standing position on 18 July 1968. The heart measures 15.4 cm, the chest 37.5 cm in diameter.

In spite of two weeks of intermittent dialysis his condition remained essentially unchanged until the 15th day after admission when he developed acute pericardial constriction which necessitated pericardiocentesis. Azotemia was later brought under control during regular hemodialysis. After successful renal allotransplantation the blood pressure and cardiac size became normal.

Autopsy findings

Eighty-five of the 125 patients in the series died; autopsy was performed in 88 and evidence of pericarditis was found in 32 (38%). In 24 patients the diagnosis had been made during life. Fibrinous or sero-fibrinous changes were found in 21. Fibrinous, or healed pericarditis with numerous synechiae was seen in 11 and in eight these changes were so marked that the pericardial cavity was completely obliterated. Calcification of the pericardium was not seen. In eight patients (9%) there was autopsy evidence of pericarditis but no pericardial rub had been heard and the diagnosis was therefore made post mortem. Severe fibrinous pericarditis with

total obliteration of the pericardial cavity was found in seven patients and pericardial fluid in one.

Pericardial fluid

This was obtained from ten patients either by pericardiocentesis during life or at autopsy. The fluid was sero-sanguinous in most cases and the volume varied from 60 to 1500 ml. Cardiac constriction was seen in one case where the pericardial cavity contained 1400 ml of sero-sanguinous fluid. Two patients demonstrated purulent pericarditis as a complication of a *Staphylococcus septicemia*. True hemopericardium was not seen (Table VI).

DISCUSSION

Pericarditis was demonstrated in 40 of 125 patients with terminal uremia (32%). This frequency of occurrence is in accordance with the findings of others (2, 3, 12). In five out of the total 40 cases of uremic pericarditis life-threatening acute pericardial constriction due to cardiac tamponade developed.

The reason why pericarditis develops in some uremic patients and not in others and likewise why effusion develops in some cases of pericarditis and not in others is unknown. In our series pericarditis was seen in patients who were severely uremic and an acute pericardial con-

Table VI Character of pericardial effusion

Autopsy and pericardiocentesis (total 10 pats.)	
Serous	3 (range 60-100 ml)
Sero-hemorrhagic	5 (range 125-1400 ml)
Hemopericardium	1
Purulent	2 (range 400-700 ml)

striction syndrome developed among the most severely uremic of these patients ($Cl_{Cr} < 2$ ml/min)

The diagnosis of uremic pericarditis is made by auscultation as the most constant symptom is the presence of a pericardial friction rub. Generally there are no subjective symptoms but some patients do complain of precordial oppression. Clear cut electrocardiographic and roentgenologic changes appear only when pericarditis is complicated by effusion.

The speed with which fluid accumulates and thus the amount of pressure produced in the pericardial cavity determines the severity of symptoms in cases of pericardial effusion. The volume of the effusion seems to be of less importance. Similarly the hemodynamic changes which determine the clinical symptoms are the result of pressure changes. Diastolic filling of the ventricles is diminished because of increased intra pericardial pressure. At the same time the diastolic pressure in the right ventricle increases and this produces a pressure increase in the right atrium and thereby an increase in the peripheral and central venous pressure. Cardiac output is temporarily maintained by an increase in frequency and by peripheral vasoconstriction but when a critical intra pericardial pressure is reached cardiac output decreases, the blood pressure falls and circulation stops. Pericardiocentesis with the aspiration of the effusion permits rapid and complete reestablishment of circulation by reducing the pressure in the pericardial cavity.

Slow development of a pericardial effusion accompanied by only a slight rise in intra pericardial pressure may be difficult to differentiate from the cardiac enlargement resulting from arterial hypertension, severe anemia and overhydration—all of which are common in terminal uremia. In two of our patients a slow accumulation of fluid in the pericardium was manifested by disappearance of the normal cardiac contours on X ray without there being any symptoms or physical signs of cardiac constriction (Table IV cases 2 and 7).

The demonstration of acute cardiac constriction in our patients was made solely on clinical observation, the rapid development of cardiac insufficiency in the course of a few hours with dyspnea, precordial oppression, engorged neck veins, hepatomegaly, falling blood pressure, de-

creasing pulse pressure and lethargy. The possibility of pericardial constriction should be borne in mind when a uremic patient with an enlarged heart suddenly develops cardiac insufficiency.

Increasing heart size with disappearance of the normal cardiac contour was demonstrated roentgenologically in four of our patients during the days immediately preceding the development of manifest cardiac tamponade. Repeated chest films are thus a helpful and important measure in the control of patients with uremic pericarditis. Characteristic electrocardiographic changes may be an aid but they are not constant and give no warning of threatening or manifest cardiac constriction. In addition measurement of the venous pressure should be a part of the routine control of patients with uremic pericarditis. The use of ultrasound in the diagnosis of pericardial effusion has recently been described (10). This new technique seems to make rapid diagnosis possible without discomfort to the patient.

In most cases uremic pericarditis provides an indication for hemodialysis; however, universal heparinization in connection with dialysis has been mentioned as a causative factor in the development of hemopericardium (1, 2, 3, 8, 9). Coagulation studies in our patients showed a prolonged plasma thrombin time which became normal on the addition of toluidine blue. This abnormality can be seen with heparin and heparin-like anticoagulants as well as in uremia. True hemopericardium was not seen in our patients.

One patient developed an acute constriction syndrome during his first hemodialysis. On pericardiocentesis 1360 ml sero-sanguinous fluid was removed and the clinical state of the patient improved. Thereafter 16 hemodialyses with universal heparinization were carried out with no complications (Table IV case 1). Six other patients with uremic pericarditis were treated with intermittent hemodialysis and universal heparinization. In one of these six patients pericardiocentesis had been performed twice because of a sero-sanguinous pericardial effusion with cardiac constriction (Table IV case 6). Hemodialysis was uncomplicated in all of these cases and in three patients the size and shape of the heart became normal. All seven patients received kidney transplants (Table IV). In this series eight patients with uremic pericarditis, including two with cardiac tamponade, have received renal trans-

plants Seven of these patients are alive one 3 years post transplant

Universal heparinization in connection with hemodialysis does not therefore seem to be contraindicated in patients with uremic pericarditis

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STUDIES ON FATTY ACID METABOLISM IN DIABETICS DURING EXERCISE

VII Plasma Glycerol Concentrations in Juvenile Diabetics during Exercise before and after Adequate Insulin Treatment

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Abstract Male newly diagnosed diabetics of the juvenile type have been examined with a short exercise test on a bicycle ergometer and the plasma concentration of glycerol was followed. As earlier shown the exercise induced a greater lipid mobilization in the diabetics in comparison with control subjects studied earlier.

The exercise test was then repeated after a period of adequate insulin therapy. At the second examination the diabetics showed a clear tendency to normalization of the plasma glycerol curve indicating that insulin therapy will decrease the abnormally high exercise induced lipid mobilization in diabetes mellitus.

Male juvenile newly diagnosed diabetics show during exercise a greater and longer persisting lipid mobilization than control subjects of the same age. This is revealed by higher concentrations in plasma both of free fatty acids (FFA) and of glycerol during and after the exercise period (2, 3). Adequate insulin treatment of the disease leads to a normalization of the plasma FFA concentrations (4).

The present study was undertaken to find out if insulin therapy also brings about a normalization of the plasma glycerol concentrations in connection with exercise. It is now well known that the plasma glycerol concentration is a better parameter of the lipid mobilization rate than plasma FFA as glycerol is not reutilized in adipose tissue for metabolic purposes (7, 10). For that reason it might be of interest to know whether the plasma glycerol level during exercise is normalized in diabetics after insulin therapy and thus whether the normalization of the FFA curve is really due to a decrease of the lipid mobilization rate.

MATERIAL AND METHODS

Five male newly diagnosed juvenile diabetics, aged 19-28 years, were selected for the study. All were diabetics of the juvenile onset type with subjective symptoms. Some clinical data of the patients are summarized in Table I. None of the patients were dehydrated or ketosacidotic when examined.

The diabetic subjects were all examined in the fasting state and at the first examination none had received insulin therapy. They were then treated with long acting insulin and re-examined when they clinically were under good control.

The examination started in the morning. After resting for 1 hour they performed graded exercise on a bicycle ergometer at a work load of 600 kpm/min for 10 min in the supine position. They were then again allowed to rest.

Blood samples were taken through an indwelling arterial catheter at intervals indicated in Table II. When not in use the catheters were filled with physiological saline. Details concerning the examination has been published previously (2).

Plasma glycerol was determined according to Laurell and Tibbling (8). The plasma FFA analyses were made according to Trout et al (11) and the blood glucose concentrations according to Marks (9).

In addition some hemodynamic data were registered but these will be published elsewhere.

RESULTS

The plasma FFA and the blood glucose concentrations during both examinations are summarized in Fig. 1. At the first examination the FFA pattern during exercise is in good accordance with earlier published results (2). At the second examination performed after adequate insulin treatment there is a clear tendency to normalization

Table I Some clinical data for the diabetic patients examined

Case	Age (y)	Height/weight (cm/kg)	Plasma creatinine	Blood pressure	Duration of symptoms (weeks)	Complications
D 1	25	177/47.5	0.8	115/75	8	0
D 2	19	179/65.0	1.10	130/75	3	0
D 3	20	184/67.4	1.10	125/90	4	0
D 4	22	177/63.1	(NPN 36)	140/80	4	0
D 5	24	183/75.0	(NPN 30)	145/90	9	0

NPN, non protein nitrogen

Table II Plasma glycerol concentrations (μ moles/l) during the experiment before insulin treatment

Case	Time																	
	Hours				Minutes													
	-2	-1½	-1	-½	1	3	5	8	11	13	15	18	25	40'	55	70	100'	
D 1	84	76	84	114	170	138	155	175	215	215	210	190	134	90	103	110	123	
D 2	76	87	94	136	148	161	190	238	269	272	286	262	182	111	77	100	107	
D 3	43	43	75	110	112	107	135	212	303	318	348	364	346	129	68	37	57	
D 4	84	75	105	99	100	107	154	178	168	404	406	387	248	80	38	31	71	
D 5	70	49	103	140	190	210	253	308	376	377	384	361	225	96	55	56	109	
Mean	71	66	92	110	134	145	174	242	306	317	327	313	227	96	68	69	93	
S.E.M.	7.6	8.2	5.7	7.9	16.1	19.3	20.9	23.5	30.3	34.4	35.6	37.6	35.5	8.8	10.9	17.6	11.4	

Table III Plasma glycerol concentrations (μ moles/l) during the experiment after insulin treatment

Case	Time																
	Hours				Minutes												
	-2	-1½	-1	-½	1	3	5	8	11	13	15	18	25	40	55	70	100
D 1	54	46	46	53	85	87	91	99	106	102	101	101	102	99	94	91	—
D 2	55	50	81	86	118	178	138	130	138	135	128	118	97	78	72	75	—
D 3	73	4	89	63	70	73	92	—	158	—	176	165	155	101	55	58	—
D 4	76	46	59	84	101	111	160	156	360	387	397	374	232	88	48	46	59
D 5	51	50	77	92	105	139	166	189	222	235	235	215	159	71	39	111	64
Mean	62	47	66	6	96	108	129	169	197	215	207	195	149	87	62	61	61
S.E.M.	5.3	1.5	7.4	7.5	8.3	12.3	16.2	34.6	44.9	64.0	52.6	49.1	44.4	5.8	9.7	10.1	2.4

of the FFA curve which agrees well with earlier published findings (4). The plasma FFA curve at the second examination is not different from that found in control subjects of the same age (2).

The plasma glycerol concentrations at the two examinations are given in Tables II and III and all values are summarized in Fig. 2. Compared with that of controls (3) the plasma glycerol curve

at the first examination is elevated before, during and for a period after exercise. This is in agreement with earlier findings (3). At the second examination each diabetic shows a tendency to normalization of the plasma glycerol curve and the mean curve for the diabetics is clearly lowered. However, the mean curve is still somewhat elevated in comparison with that of controls studied earlier (3).

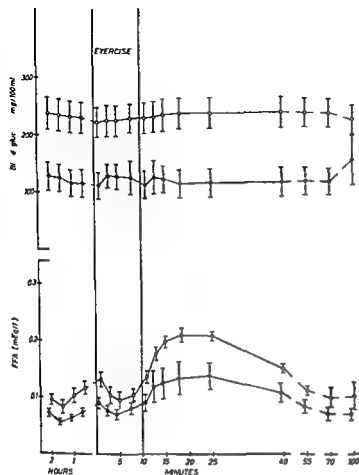


Fig 1 Plasma FFA and blood glucose concentrations before (O) and after (●) in sulin treatment. Mean \pm standard error of the mean.

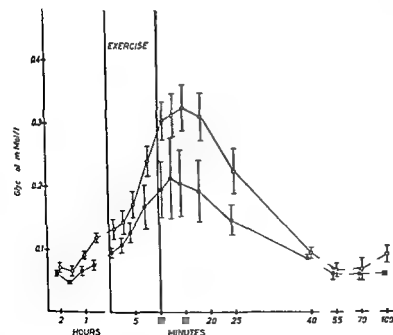


Fig 2 Plasma glycerol concentrations before (O) and after (●) in sulin treatment.

DISCUSSION

One of the striking features in the deranged fat metabolism in diabetes mellitus is the increased lipolysis in adipose tissue and the consequently augmented mobilization of fatty acids and glycerol to plasma. As stated in the introduction glycerol cannot be reutilized in adipose tissue and thus is the best indicator of the rate of the lipid mobilization (7-10). It is now well known that the plasma glycerol concentration is elevated at rest in diabetics (3-5). Exercise stimulates the lipid mobilization and leads to higher plasma concentration of glycerol in control subjects (1-6) and to abnormally high plasma levels in untreated diabetics (3). This is presumably due to a more lightly initiated lipolysis in the adipose tissue in diabetes. Judging from the present study adequate insulin therapy appears to decrease the abnormally high exercise induced lipid mobilization in diabetes mellitus.

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ACCIDENTALLY TRANSMITTED INFECTIOUS MONONUCLEOSIS

Report of a Case

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Abstract A case report is given of infectious mononucleosis (IM) accidentally transmitted to a patient by transfusion. The donor two days after giving 450 ml citrated blood experienced malaise and IM was diagnosed with a typical clinical and haematological course. The citrated blood was stored for three days before the donation. The transfused patient three weeks later developed a febrile syndrome with general adenopathy and the haematological features of IM. The diagnosis of IM was supported by a positive horse-cell slide agglutination test (EMNT test).

By definition infectious mononucleosis (IM) is a subacute or acute infection which is characterized by fever and a distinctive lymphocytosis and frequently by enlargement of cervical and other lymphatic glands or by a sore throat or both (25).

The etiology of IM is unknown. Recently a virus called the Epstein Barr (EB) virus has been discovered in patients with mononucleosis; it is a herpes-like virus that has been associated with Burkitt's lymphoma cells (12). In each of 29 patients with IM the development of antibodies against EB virus was demonstrated by Niederman et al (17) by an indirect immunofluorescence test with a virus-bearing cell line (EB 3) derived from a Burkitt lymphoma. These antibodies absent in pre-illness serum specimens usually appeared early in the disease, rose to peak levels within a few weeks and remained at relatively high levels during the convalescence. These and other observations indicate that EB virus or a closely related one is the etiologic agent of IM. The etiology of the infection, however, is still a subject of debate (6, 7).

IM though it has been known previously by

other names was considered to be a curiosity when Sprunt and Evans (23) first coined the name almost 50 years ago. There is evidence that the incidence of the disease is increasing (24) and today it is really a common disease. At present IM appears to be less epidemic in character and more sporadic. Transmission is probably by direct presumably intimate contact with infected persons (14).

Attempts to transmit the disease to laboratory animals by various inocula have met with negative or equivocal results. Human volunteers transfused with blood from patients with IM have on a few occasions developed suggestive clinical evidence of the disease. Only in a single case has an obviously successful experimental transmission been carried out in this way (28). It is noteworthy that the literature describes only two cases in which a patient accidentally acquired IM as a consequence of a blood transfusion from a donor with this disease (3, 18). This statistic appears singularly unimpressive in view of the incidence of the illness.

We have had a patient in whom IM occurred after a blood transfusion from a donor who experienced the first symptoms of IM two days after the transfusion.

CASE REPORTS

Patient 1

I. S. B. A 21-year-old female student was admitted to the Medical Department Sentralsykehuset Akershus on March 13, 1968. She was previously healthy and had been a blood donor for one year. She had last given blood on February 11 of the same year. During the very first days of March she noticed oedema of the eye lids.

and experienced a general malaise, nausea, anorexia and severe headache.

Status on admission March 13 1968 General condition good. Temp 38.2°C. The patient complained of headache, slight anginous throat trouble and fatigue. There was no rash or jaundice. The examination revealed moderate reddening of the pharynx without palatal enanthema. Along both sterno-cleido-mastoids in each axilla and inguinal region a number of enlarged lymph nodes were present. The lymph nodes ranged from about bean up to hazel nut-size.

Internal organs Slightly epigastric tenderness was noted during palpation of the abdomen. The spleen was easily palpable two fingerbreadths below the left costal margin not tender. Liver not palpable.

Laboratory findings on admission were as follows Urine and spinal fluid no abnormality. Hb 118 g/100 ml, white cell count 16700/mm³ with 78% atypical lymphocytes. They were mostly claved as Type III by Downey (4). Blood platelets 147000/mm³. ESR mm/h serum glutamic pyruvic transaminase (SGPT) 300 units alkaline phosphatase 108 mU (Bessy Lowry) units.

The horse-cell slide agglutination test (EMNI test) was positive. The tests for sheep red cell agglutinins according to Paul and Bunnell were positive and showed a titre of 1/768 in sera after absorption with guinea pig kidney.

Wasserman Kahn and MBR II tests were negative. A diagnosis of IM was made by the characteristic clinical and laboratory features. The hospital course was uneventful; the patient was discharged on March 21 1968 and later followed up for half a year. There was a typical clinical and haematological course.

During her stay in hospital we checked the information given by Patient 1 that she had donated blood two days before she experienced the first symptoms of her illness. By coincidence the transfusion turned out to have been carried out in the Surgical Department of our hospital. The transfused patient is hereafter referred to as Patient 2.

Patient 2

M A, a previously healthy dressmaker aged 18 had been admitted to the Surgical Department on March 1 due to a traffic accident.

Status on admission March 1 1968 The patient complained of pains in the chest and in her left thigh. Further examination revealed fractures of the left clavicle and the tenth right rib and some laceration of the soft tissue of the left thigh. The temperature was normal. There was no adenopathy.

Internal organs The liver and the spleen were not palpable.

Laboratory findings on admission were as follows Hb 107 g/100 ml, white cell count 6000/mm³ with 16% lymphocytes, 2% monocytes, and 82% granulocytes showed a moderate shift to the left.

On her first day in hospital she received 400 ml stored blood from Patient 1 who we learnt 14 days later had infectious mononucleosis. Thus the blood had been stored for three days before the transfusion.

On March 10 Patient 2 was more closely examined in the Medical Department. She was at that time run-

ning a variable pyrexia with temperature from 37.7 to 38.2°C. She complained of general malaise and a tired feeling which could be a result of the accident. She had, however, had a mild sore throat for two days and on examination pharyngeal injection without exudate was found. There was no eyelid oedema, no exanthema, no palatal enanthema. General adenopathy was present. Of the cervical glands the posterior chains were palpable down to the clavicle; the individual glands were of the size of beans. The edge of the liver could be felt below the right costal margin, non-tender. The spleen was not palpable.

Laboratory investigations On March 20 the percentage of Hb was 123 g/100 ml, ESR 43 mm/h, white cell count 12,400/mm³ with 42% neutrophils, 58% mononuclear cells. Most of the mononuclear cells were atypical lymphocytes (43% of the total number of white cells). The abnormal cells we encountered were mostly claved as Type I and II by Downey; a few cells with the appearance of Type III were also seen. Serum glutamic pyruvic transaminase (SGPT) was 22 units, thymol 1.6 MacLagan units.

The horse-cell slide agglutination test (EMNI test) was distinctly positive and was repeated on March 21 and again found indisputably positive. The tests for sheep-cell agglutinins according to Paul and Bunnell were negative and remained so throughout the time of observation.

Tests for toxoplasmosis, the Brucella agglutination test, and tests for cytomegal virus were all negative. Wasserman Kahn and MBR II test were also negative.

The clinical picture gradually improved. On April 1 the patient had no complaints which unambiguously could be referred to the transmitted IM. The fatigue, however, was still present. The liver could not be felt; the general adenopathy was easily demonstrated although in recession.

On April 1 examination of the peripheral blood revealed a relative lymphocytosis of 41%. The number of atypical lymphocytes amounted to 74% of the total number of white cells (6100/mm³); cells of Downey Type I were now dominant. The EMNI test was now positive.

Blood films examined throughout the month of April showed a decreasing number of mononuclear cells. In most of the blood films, cells were seen which were intermediate in morphology between normal lymphocytes and the atypical cells, and it was therefore possible to estimate only the approximate proportion of atypical mononuclear cells in the total white population. The range of atypical cells was 610 to 1100/mm³ and the proportion of these cells in the total lymphocyte count averaged 40%. In July the blood picture was not analyzed.

DISCUSSION

Many attempts have been made to transmit IM to animals and humans inoculated with whole blood, serum, excised lymph nodes, faeces and gargle washings from patients with the disease.

(18 9 16 27) Convincing evidence of successful transmission of this disorder has been missing in almost all these experiments. Two attempts only may be acceptable as instances of successful transmission to man—that of Sohier et al (21) and particularly that of Wising (28). In a critical review of transmission experiments with IM Hoagland states that he is inclined to believe that Wising's experiment can be regarded as successful (14). In both cases the experimental transmission of IM was attempted with whole blood as inoculum.

Accidental transmission of IM by blood transfusions seems to be very rare as only two cases are reported in the literature (3 18). De Vos and Kuipers (3) in 1951 reported that they involuntarily transmitted IM by transfusing 400 ml of blood into a patient. The latter experienced fever 21 days later, splenic enlargement was observed and lymphocytes constituted 88%. 42 days after transfusion many atypical lymphocytes were present. Thirty-four days after transfusion the heterophil antibody titre was 1:128. The donor one week after giving blood experienced malaise and an examination revealed a mononucleosis. Paloheimo and Halonen in 1965 (18) described a mononucleosis-like syndrome in a 15-year-old girl with congenital heart disease. The patient received a blood transfusion from pooled blood of nine blood donors; extracorporeal circulation was not used. Five to six weeks later the "typical" syndrome with general malaise, fever, lymphadenopathy, lymphocytosis with atypical lymphocytes occurred. The Paul and Bunnell test was negative. One of the blood donors had had IM.

Circulating atypical mononuclear cells often unassociated with fever and lymphocytosis have been observed in the postoperative period in patients who have had open heart surgery. Forster (10) reported these findings in 30 of 180 patients. It has been suggested that post-transfusion mononucleosis in such cases may represent an immune response to viable lymphocytes contained in freshly donated blood, essentially a graft of living lymphocytes (5). In some cases however it has been proven that the patient accidentally has acquired a cytomegalovirus infection (for review see Paloheimo et al (19)).

Our patient acquired an illness with the clinical criteria of a mild attack of IM about three weeks after having received a transfusion with 450 ml

from a donor who two days after the donation experienced the first symptoms of typical IM. We regard the illness of our patient to be a case of post-transfusion mononucleosis due to transmission of the (unknown) causative agent of IM. A rise and fall in abnormal lymphocytes was observed during the illness and a repeated horse cell slide agglutination test (EMNI test) was indisputably positive 20 and 22 days respectively after the transfusion. The EMNI test however was negative five weeks after the transfusion.

The incubation period of naturally acquired IM is considered to be about 33–49 days (13). A case report of IM occurring 21 days after blood transfusion shows that the incubation period might be shorter when the disease is transmitted in this way (3). This last experience is in accordance with the interval between the transfusion and the state of illness in our patient.

Although the presence of abnormal lymphocytes is a characteristic finding in IM, it is not pathognomonic. Numerous atypical lymphocytes are frequently observed in infective hepatitis and similar cells have also been described in brucellosis, acquired toxoplasmosis, cytomegalovirus infection and allergic conditions (for review see Penman (20)). A diagnosis of infective hepatitis could be excluded in our case. On the contrary the normal liver function tests, as in our patient, are rather unusual in patients with IM. By the improved tests now available it has been shown that the function of the liver is impaired in 90 to 100% of patients with IM. We have however occasionally observed patients with (seropositive) IM in whom serial blood samples drawn had a normal amount of serum glutamic pyruvic transaminase (SGPT). This experience is in accordance with that of Hoagland (14) though we admit that an increase of this serum enzyme is the rule during the course of IM. In a recent report by Trostman and With (26) the thymol turbidity test and the serum glutamic-oxaloacetic transaminase (SGOT) were found to be positive in the majority but not in all cases of IM during the acute febrile stage.

Acquired toxoplasmosis, brucellosis and cytomegalovirus infection could be excluded in our patient.

Until the aetiology of IM is ultimately defined its diagnosis must necessarily be based on a composite of clinical, haematological and serological

and experienced a general malaise nausea, anorexia and severe headache

Status on admission March 13 1968 General condition good Temp 38.2°C The patient complained of headache slight anxious throat trouble and fatigue There was no rash or jaundice The examination revealed moderate reddening of the pharynx without palatal enanthema Along both sterno-cleido mastoids in each axilla and inguinal region a number of enlarged lymph nodes were present The lymph nodes ranged from about bean up to hazel nut size

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Patient 2

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Status on admission March 1 1968 The patient complained of pains in the chest and in her left thigh Further examination revealed fractures of the left clavicle and the tenth right rib and some laceration of the soft tissue of the left thigh The temperature was normal There was no adenopathy

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ning a variable pyrexia with temperature from 37.7 to 38.2°C She complained of general malaise and a tired feeling which could be a result of the accident She had however had a mild sore throat for two days and on examination pharyngeal injection without exudate was found There was no eyelid oedema no exanthema no palatal enanthema General adenopathy was present Of the cervical glands the posterior chains were palpable down to the clavicle the individual glands were of the size of beans The edge of the liver could be felt below the right costal margin non tender The spleen was not palpable

Laboratory investigations On March 20 the percentage of Hb was 12.3 g/100 ml, ESR 43 mm/h white cell count 12 400/mm³ with 42 neutrophils 58% mononuclear cells Most of the mononuclear cells were atypical lymphocytes (43% of the total number of white cells) The abnormal cells we encountered were mostly classed as Type I and II by Downey a few cells with the appearance of Type III were also seen Serum glutamic pyruvic transaminase (SGPT) was 22 units thymol 16 MacLagan units

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Tests for toxoplasmosis the Brucella agglutination test, and tests for cytomegal virus were all negative Wasserman Kahn and MBR II test were also negative

The clinical picture gradually improved On April 1 the patient had no complaints which unambiguously could be referred to the transmitted IM The fatigue however was still present The liver could not be felt, the general adenopathy was easily demonstrated although in recession

On April 1 examination of the peripheral blood revealed a relative lymphocytosis of 41 The number of atypical lymphocytes amounted to 24% of the total number of white cells (6100/mm³) cells of Downey Type I were now dominant The EMNI test was now negative

Blood films examined throughout the month of April showed a decreasing number of mononuclear cells In most of the blood films cells were seen which were intermediate in morphology between normal lymphocytes and the atypical cells and it was therefore possible to estimate only the approximate proportion of atypical mononuclear cells in the total white population The range of atypical cells was 610 to 1100/mm³ and the proportion of these cells in the total lymphocyte count averaged 22 In July the blood picture was normalized

DISCUSSION

Many attempts have been made to transmit IM to animals and humans inoculated with whole blood serum excised lymph nodes faeces and gargle washings from patients with the disease

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ATRIAL TACHYCARDIA WITH BLOCK

A Clinical Study of 40 Episodes in 32 Patients

Leif Hillestad

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Abstract A study has been made of 40 episodes of atrial tachycardia with block in 32 patients observed during the years 1965 through 1968.

Mitral valve disease was the most frequent underlying disease while aortic valve disease was nearly absent. The rhythm disorder produced significant deterioration in a majority of the patients and precipitated severe complaints even in persons with a normal heart. Most episodes ran a continuous course the paroxysmal variety being rather infrequent. It is therefore suggested that the arrhythmia is termed atrial tachycardia with block and the prefix "paroxysmal" added only when distinct attacks are observed.

Only three patients died all from their underlying disease. This mortality is probably the lowest ever recorded in series of this rhythm disturbance.

The diagnostic criteria largely proved satisfactory but differentiation against atrial flutter remains controversial.

Digitalis poisoning was possibly present in only nine of the 40 episodes. Contributory causes including hypokalemia were not demonstrated. Nor was any specific heart rhythm found to be the prime predecessor of atrial tachycardia with block.

Treatment consisted of discontinuance of digitalis and peroral administration of potassium chloride. Some 3-4 days thereafter electroconversion was undertaken. This treatment proved to be safe, reliable and effective and superior to treatment with various antiarrhythmic agents.

Atrial tachycardia with block is easily overlooked but important to recognize. Firstly because it often produces serious clinical deterioration. Secondly because it may be misinterpreted as atrial flutter which calls for increased dosage of digitalis. Atrial tachycardia with block however may be a sign of digitalis poisoning and a further increment thus lead to disastrous results (7, 10). Thirdly atrial tachycardia with block is unique in being able to present as a bradyarrhythmia.

This report is a study of atrial tachycardia

with block its diagnostic criteria its varying clinical pictures and its relationship to treatment with digitalis glycosides.

MATERIAL

The series comprises 40 episodes of atrial tachycardia with block in 32 patients admitted during the years 1965 through 1968. This gives a mean number of ten episodes discovered among some 7000 ECGs yearly. The frequency did not rise during the observation period. The arrhythmia occurred on three separate occasions in two patients, and on two separate occasions in four patients.

The diagnosis of atrial tachycardia with block was made according to the criteria put forward by Lown et al (10): an atrial rate between 150-300 per min, an isoelectric baseline between the P waves in all leads and the existence of atrioventricular block beyond simple prolongation of the P-R interval.

An important diagnostic aid in this study was obtained from the esophageal lead: the use of which caused minor discomfort even in critically ill patients.

In this series atrial tachycardia with block (AT with block) did not consistently appear in paroxysms. Consequently the prefix "paroxysmal" has been omitted in the following.

RESULTS

The total series (Table I) showed mitral valvular disease to be the prevalent underlying disorder while the absence of aortic valve disease was unexpected as these two disorders are evenly distributed at the department.

Among the five men with other heart diseases were the only one with aortic valve disease, another treated with closure of a secundum type of atrial septal defect, one with an isolated pulmonary incompetence, one with a sino-atrial and

Table I *Distribution of sex, age and underlying disease in the series of atrial tachycardia with block*

Sex	No	Mean age	Mitral valve dis	Atheroscl heart dis	Other heart dis
Men	12	61	1	4	5
Women	20	53	11	2	6
Total	32	56	12	6	11

Table II *Frequency distribution of atrial and ventricular rates in 40 episodes of atrial tachycardia with block*

Rate per min.	Atrial	Ventricular
<100	—	19
100-149	3	20
150-199	13	1
200-249	20	—
>250	4	—
Mean	213	103

one with a complete atrioventricular block. The latter two both required treatment with an artificial pacemaker.

Among the corresponding six women there were two who had been operated on for atrial septal defect of secundum type; one suffered from amyloidosis, one from a sino atrial and one from a complete atrioventricular block while one suffered from a thyrotoxic cardiomyopathy.

In two patients, one of each sex, no underlying disease could be demonstrated.

The heart volume was normal in seven patients and considerably enlarged in 18. In the remaining seven patients the heart was markedly enlarged, the calculated heart volume exceeding 1000 ml per sq m body surface.

The clinical effects produced by AT with block usually were apparent. A majority of the patients worsened sometimes critically when under the influence of the arrhythmia. Its adverse effects could best be assessed by the clinical improvement following its abolition.

This study showed AT with block to be a rhythm disorder which in principle followed a continuous course. Only three patients experienced distinct bouts or paroxysms. In the remainder AT with block tended to persist for weeks, months or years until interrupted by treatment. One

woman in this series suffered from the arrhythmia continuously for six years. In such long standing cases the atrioventricular block may vary and produce different heart rates. This variation is however not to be mistaken as attacks.

Only three of 32 patients died in the present series, the mortality thus being unusually low. A 37 year-old woman died from amyloidosis. Another woman aged 62 with advanced mitral valve disease had attacks of AT with block accompanied by severe anginal pain. Discontinuation of digitalis caused disappearance of the arrhythmia, intermediate occurrence of nodal rhythm and ultimately of sino atrial block requiring treatment with artificial pacemaker. The patient died during an attack of anginal pain when under preoperative care for implantation of an artificial mitral valve. Necropsy revealed a calcified mitral ostium, coronary artery obstructions and fibrosis around the conduction system. A man aged 58 died from advanced atherosclerotic heart disease.

The above patients all died from their primary disease but the rhythm disorder seemed to add markedly to the deterioration leading to death.

The diagnostic criteria proved largely satisfactory (Table II). The highest and lowest atrial rate recorded was 112 and 273 respectively. Nearly half of the episodes had ventricular rates

Table III *The frequency of various types of atrioventricular block in 40 episodes of atrial tachycardia with block*

Type of A-V block	No
2:1	2
3:1	6
4:1	2
Wenckebach	3
Varying	4
Complete	3

Table IV *The rhythms immediately preceding 23 episodes of atrial tachycardia with block*

Preceding rhythm	No
Sinus rhythm	9
Atrial fibrillation	9
Atrial flutter	1
Varying rhythms	4

11/4 1966

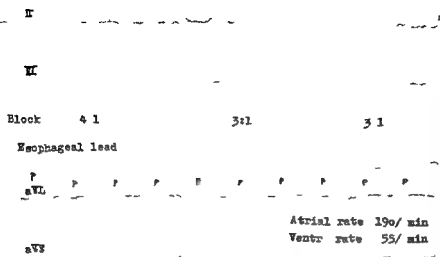


Fig 2 Case 2 This ECG was initially interpreted as sinus bradycardia, but the esophageal lead disclosed atrial tachycardia with 3:1 and 4:1 atrioventricular block and the corresponding low ventricular rate. A low pulse rate does not exclude the presence of atrial tachycardia with block.

been used in constant dosage for months or years when AT with block appeared for unknown reasons. A total of nine episodes were without relationship to the use of digitalis.

Slight hypokalemia was observed in one patient only. Other contributory causes such as trauma, copious diuresis, concomitant disease or grave clinical deterioration (10) could not be demonstrated.

The rhythm present at the outbreak of AT with block could be safely determined in 23 episodes (Table IV). It seems clear that no specific rhythm can be regarded as the prevalent predecessor of AT with block.

The treatment of AT with block in this study consisted in immediate discontinuance of digitalis and peroral ingestion of potassium chloride. Electroconversion was then to be done 3-4 days later. This approach covered a twofold purpose. Firstly, a possible digitalis poisoning was allowed to disappear. Thus spontaneous conversion to sinus rhythm occurred in six episodes in the course of 2-3 days. Secondly, the use of various drugs with several unwanted side effects was avoided. Instead, the patients were exposed to the safe, simple and effective procedure of electroconversion. Thus 33 episodes were successfully converted while only one episode proved resistant to this treatment.

In some cases of possible digitalis poisoning, potassium was administered intravenously with

out the immediate effect noted by others (4, 10). Other antiarrhythmic agents were given a trial also, but none of them proved consistently effective.

It is to be added that the great majority of the patients treated were dismissed on continued digitalis. So far, the rate of relapse has been negligible.

CASE REPORTS

In the sequel, some illustrative case histories from patients suffering from AT with block are presented.

Case 1 woman 57 years old (Fig 1). In the last six years, palpitations accompanied by dyspnea, severe angina pectoris and syncope. Treated with digitalis for five years without effect. Digitalis discontinued 13 months prior to the admission. The first ECG (8/8/1968) was interpreted as sinus rhythm with atrial premature beats, but close inspection aroused suspicion of the presence of AT with block, which was verified by means of an esophageal lead. A peculiar repetitive second degree block could be observed. Further clinical examination did not reveal coexistent or underlying disease. Sinus rhythm was restored by means of electroconversion and the patient dismissed on quinidine treatment.

Case 2 man 50 years old (Fig 2). For two years, severe angina pectoris treated with digitalis, anticoagulants and nitroglycerine without effect. On admission, the first ECG (11/4/1966) was initially interpreted as sinus bradycardia. However, an esophageal lead disclosed an AT with block with a remarkably low ventricular rate. Although no signs of digitalis poisoning were present, this agent

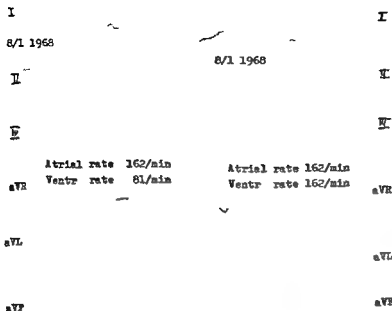


Fig 3 Case 3 The left tracing shows atrial tachycardia with regular 2:1 block, which in the right tracing disappears in response to an exercise test

was discontinued. After three days spontaneous conversion to sinus bradycardia took place. Further clinical examination gave no evidence of underlying disease. Coronary heart disease could not be demonstrated by objective means. The heart was of borderline size. The patient was dismissed on treatment with digitalis and quinidine.

Case 3 woman 21 years old (Fig 3). In the last year troubled by attacks of uncomfortable palpitations accompanied by pain, fear and dizziness. Clinical examination was unrevealing. The ECG during attacks showed

AT with block interrupted by periods of sino atrial block and periods of atrial fibrillation. During AT with 2:1 block a Master's two step test was done. The block thereby vanished. Digitalis, quinidine and beta adrenergic blocking agents failed to prevent the bouts of AT with block.

Case 4 woman 46 years old (Fig 4). About four weeks after mitral re-commissionectomy sudden deterioration with dyspnea and chest pain. ECG (11.6.1964) revealed AT with block most distinctly seen in V. This block exhibited Wenckebach's periods. Sinus rhythm was

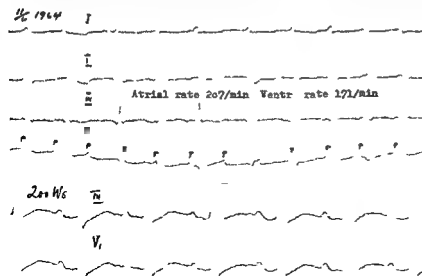


Fig 4 Case 4 Upper tracing shows atrial tachycardia with distinct, small P waves and isoelectric baseline intervals in lead V. The block exhibits Wenckebach periodicity. Lower tracing demonstrates sinus rhythm obtained by electroconversion. Note the identical shape of P waves in the two tracings.

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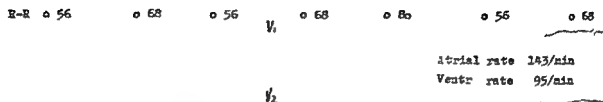


Fig 5 Case 4 Atrial tachycardia with second degree block of a peculiar appearance with repetitive periods unlike those of Wenckebach.

restored by means of electroconversion. Note the resemblance of the P waves in the two rhythms. This should be so because the impulse focus in AT with block is known to lie in the near vicinity of the sinus node (1).

Case 5 woman 57 years old (Fig. 5). Operated upon with transplantation of an artificial mitral valve. Some

weeks after surgery occurrence of AT with block. This block showed a peculiar periodicity over five beats alternating with shorter periods of 2:1 and 1:1 block. Discontinuance of digitalis, treatment with potassium, quinidine, procainamide and electroconversion failed to abolish the arrhythmia.

Case 6 woman 69 years old (Fig. 6). For some years treated for paroxysmal atrial flutter. Admitted for troublesome tachycardia on March 29, 1966. The ECG revealed signs typical of AT with 2:1 block and was described accordingly. Sinus rhythm was restored by means of electroconversion and the patient dismissed on continued digitalis. Readmitted on November 14, 1966 for tachycardia. The ECG showed atrial and ventricular rates identical with those of the ECG from March 9. However, this time the ECG was interpreted as atrial flutter. This was due to the presence of negative P waves and absence of isoelectric baseline in lead V₁, although typical flutter oscillations were nowhere to be seen in the whole ECG. Again sinus rhythm was restored by direct countershock.

Comparison of lead V₁ of the three ECGs shows the P waves on March 29 to be very like those produced by the sinus node on November 18, while those from November 14 are different from the latter.

All these findings would support the usual diagnosis of AT with block on March 29 and atrial flutter on November 14. Experimental data indicate that the ectopic focus in AT with block lies in the superior part of the atrium near the sinus node (1). In contrast, the ectopic focus in atrial flutter is located low in the atrium.

COMMENTS

This study provides some additional information on atrial tachycardia with block. Firstly, it makes

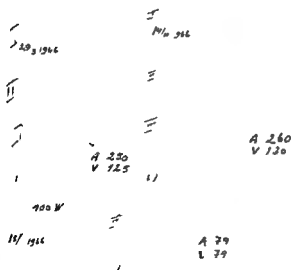


Fig 6 Case 6 The upper tracing shows two ECGs from the same patient on various occasions. The atrial and ventricular rates are identical. The left tracing was interpreted as atrial tachycardia with block, the right tracing as atrial flutter. Lower tracing shows sinus rhythm obtained by means of electroconversion.

clear that this arrhythmia is not confined only to elderly patients with serious heart disease (4 10) but also occurs in young patients and in persons without demonstrable disease at all. Mitral valve disease was the prevalent underlying disorder in the present series. For unknown reasons aortic valve disease showed a corresponding absence.

Secondly this series showed AT with block to appear mainly in a continuous form and seldom in attacks. Similar observations have been made by others (11) and a case of 25 years continuous duration has been reported (12). It is suggested that the prefix "paroxysmal" in AT with block should only be used in the presence of distinct attacks.

It is obvious from this study that the adverse effects produced by AT with block cannot be ascribed to a high ventricular rate (11) because half of the episodes showed ventricular rates below 100 per min. Additionally a man with a pulse rate of 55 suffered from severe angina pectoris. Probably the atrial rate and the inefficacy of the atrial systole play a greater role in the production of symptoms.

The mortality in the present report was less than 10% in contrast to figures from 28 to 58% in other series (4 6 10 11). Deaths due to digitalis poisoning were not encountered but have been noted by others (7 10).

The diagnostic criteria for AT with block as laid down by Lown et al (10) provide a good coverage of the subject. Still controversy may arise from differentiation between AT with block and atrial flutter. It seems wise to regard a diagnosis of AT with block as dubious if the atrial rate exceeds 270 per min even when all diagnostic criteria are satisfied. In practice this differentiation is not vitally important. The problem is easily solved by discontinuing digitalis and applying a direct countershock some days thereafter. This treatment is equally effective in both arrhythmias.

The present study also revealed a type of block not previously described: with repetitive periods over a longer range of heart beats. Furthermore it showed the second degree block to disappear in response to exercise and general anesthesia. This may explain that the presence of a continuous type of AT with block may present with intermittent beats which virtually are full and are due only to abolition of the atrioventricular

block in response to exercise or augmented sympathetic stimuli of other origin.

Coexistence of sino atrial block and AT with block has been observed in a case reported by Laake (9). In this series two similar cases were seen one of them being so serious that implantation of an artificial pacemaker became necessary.

The etiology of AT with block is unknown but poisoning with digitalis has been regarded as a chief cause in published series (4 6 10). This view has been disregarded by some authors who have noted only beneficial effects from treating all cases of this rhythm disorder with digitalis (8 11). According to the present study the etiologic role played by digitalis has probably been overestimated in the past. However digitalis poisoning has always to be considered as further poisoning has had disastrous effects (7 10). On the other hand prolonged withdrawal of digitalis is to be avoided because a majority of patients suffering from AT with block also suffer from heart failure.

Both these requirements are met by the treatment of AT with block as employed in the present series. Discontinuance of digitalis administration of potassium chloride by the oral route and application of electroconversion after some few days represent the constituents of a safe simple and effective therapy. It is followed by prompt reinstitution of digitalis treatment. This kind of therapy has been effective where other therapy has failed (3). However treatment with diphenylhydantoin (2), propranolol (5), quinidine or procainamide (4) may be justified in certain circumstances.

This study also proved that no specific heart rhythm preceded the commencement of AT with block. If so the outbreak of AT with block could have been foreseen and possibly prevented. However AT with block can follow sinus rhythm as well as all the commonly occurring rhythm disorders in patients with heart disease.

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EFFECT OF APTIN A β ADRENERGIC BLOCKING AGENT IN ARTERIAL HYPERTENSION

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Abstract The antihypertensive effect of a new beta adrenergic blocking agent Aptin has been studied in a group of 17 patients with moderate arterial hypertension. The trial was carried out using a conventional double blind cross-over design.

A significant reduction of 18 mm Hg in the systolic blood pressure at rest was recorded for the entire group. The corresponding decrease of 7 mm Hg in the diastolic pressure was not significant. During exercise a more pronounced reduction was recorded than at rest. The correlation found between the reduction in systolic blood pressure and heart rate during exercise during the Aptin treatment was probably significant.

The antihypertensive effect of Aptin was considered as moderate. The drug may be of therapeutic value in combination with other antihypertensive drugs. No significant adverse effects or changes in results of laboratory tests were noted during the Aptin treatment.

effect of Aptin was examined on patients at rest as well as during exercise.

MATERIAL

The material comprised 17 patients, six men and six women between the ages of 21 and 55. They were all previously subjected to an examination at the Medical Department. Their arterial hypertension, which was of a moderate degree, had been recognized for at least 3 months and maximally for 20 years. The clinical diagnoses are given in Table I. Case K. E. had a stenosis of his right renal artery without any demonstrable reduction of the renal function. With one exception all had hypertensive eye ground changes. Some anthropometric data are given in Table I.

METHOD

The trial was carried out using a double-blind cross-over design. The systolic and diastolic blood pressures were recorded indirectly. The blood pressure cuff was applied on the patient's right upper arm. Diastolic pressure was recorded at the muffling of the Korotkoff sounds. Every second week the blood pressures were recorded in the supine position after 10 min rest. After an initial control period of 2 weeks without treatment all patients received placebo for a further 2 weeks. Thereafter followed two treatment periods of 12 weeks with placebo and Aptin respectively in a randomized order. All tablets were identical in appearance and taste and they were given four times daily. Dosage of Aptin was commenced at 50 mg q.d. and each dose was increased by 5 mg after 2 weeks and by another 5 mg after 4 weeks.

At the end of these 12 week periods all patients performed a standardized work test on a bicycle ergometer according to Sjöstrand-Wahlund. The physical work capacity at a pulse rate of 170 beats/min (W_{170}) was calculated in the conventional way by interpolation or extrapolation (cf. 1). Hemoglobin, whole blood count,

The favourable effects of beta adrenergic blocking therapy in angina pectoris and cardiac arrhythmias have been documented in several clinical trials.

During recent years an antihypertensive effect has been indicated during such therapy in patients with arterial hypertension (3, 4, 5, 6, 8, 11). The reduction of the systolic blood pressure is twice as great as that of the diastolic according to Waal (11). The antihypertensive effect of the beta adrenergic blocking agents has usually been considered as moderate. There are different opinions concerning their therapeutic value in arterial hypertension.

The antihypertensive effect of Aptin, a new beta adrenergic blocking agent with a weak sympathomimetic effect, has been studied on outpatients with moderate arterial hypertension. The

Table I Some anthropometric and pre therapy data of 12 patients with arterial hypertension and the clinical diagnoses

Case	Sex	Age (y)	Weight (kg)	Radiological heart volume (ml/m BSA)	Fundus hyper tonicus	Heart rate at rest (beats/min)	Blood pressure (mm Hg)	W_{170} (kpm/min)	Clinical diagnoses
E N	♀	49	83	440	II	93	220/130	410	Hypertonia ess benign c mb cordis
B L	♂	46	88	420	I	81	185/125	670	Hypertonia ess benign
W E	♂	50	79	390	II	95	210/125	760	Stenosis art ren dat c hypertonia
J H	♂	44	79	490	II	54	175/95	1530	Hypertonia ess benign
E J	♀	51	52	500	I	89	180/105	870	Hypertonia ess benign
A G	♂	45	76	350	II	90	200/105	520	Hypertonia ess benign
B P	♂	43	72	520	II	70	185/120	1060	Hypertonia ess benign c mb cordis
G N	♀	54	71	350	II	100	195/105	530	Hypertonia ess benign
S L	♀	21	53	260	0	88	160/100	550	Hypertonia ess benign
B N	♂	29	65	420	I	104	155/100	580	St p coarctatio aortae oper + hypertonia ess benign
S E	♂	55	80	520	II	93	155/100	900	Hypertonia ess benign c mb cordis
V W	III	59	335	335	I	81	205/115	500	Hypertonia ess benign

Liver function tests, electrolytes, electrophoresis, and serum-creatinine were checked for all patients during the trial. Non parametric statistics were used for testing the significance of difference and correlation (9).

RESULTS

Blood pressure at rest

During the pre treatment period the blood pressure values for the entire group were 187/120 and 182/125 mm Hg respectively. At the third examination when the patients had taken the placebo for 2 weeks the corresponding values were 181/109. The latter were chosen as control values.

After 12 weeks on Aptin the systolic blood pressure was reduced in ten out of 12 patients and the diastolic blood pressure in seven. The mean pressures were 162/104 mm Hg at the end of the period. There was usually a successive reduction in the pressure during the Aptin treatment (Fig. 1). No decrease in the systolic or diastolic blood pressures occurred during the placebo period as compared to the control values. The mean values at the end of this period were 180/111 mm Hg.

The difference of 18 mm Hg between the systolic blood pressure at the end of the Aptin and placebo periods is statistically significant ($p < 0.01$). The corresponding decrease of 7 mm Hg in the diastolic pressure is not significant.

There was no correlation between the reduction in systolic pressure during the period of Aptin treatment and the corresponding reduction in the heart rate at rest.

Blood pressure during exercise

The mean systolic blood pressure during heavy exercise (mean heart rate 165 beats/min) for the entire group was 264 mm Hg at the end of the placebo period. The corresponding pressure recorded during work at the same work loads at the end of the Aptin period was 236 mm Hg. The difference of 28 mm Hg is statistically significant ($p < 0.01$). During the latter period 11 of 12 patients had a reduced pressure.

The correlation found during the Aptin treatment between the reduction of the systolic blood pressure and the reduction in heart rate during exercise at the same work load and in relative

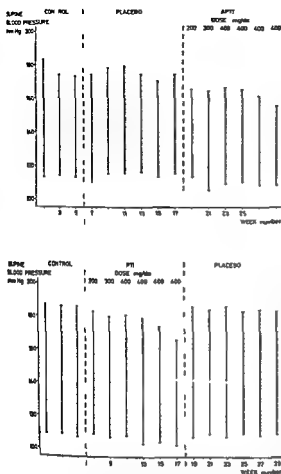


Fig 1 Mean systolic and diastolic blood pressures from a double-blind cross-over trial of Aptin in a group of 12 patients with moderate arterial hypertension

steady state was probably significant ($p < 0.05$). No such correlation was noted concerning increments in W_{170} and blood pressure.

No adverse effects that could be attributed to Aptin were reported by the patients during the trial nor were there any significant changes in laboratory findings.

DISCUSSION

The reduction in the blood pressure at rest during treatment with Aptin in the present group of patients with moderate arterial hypertension is in magnitude similar to that found by Tibblin and Ablad (10). Further the reduction in pressure after Aptin is about equal in magnitude to that reported for propranolol by Richards (6) and Waal (11).

The antihypertensive effect of Aptin was more pronounced during exercise than at rest. Similar results have previously been observed in acute tests with propranolol (8).

The reduction of the blood pressure during beta adrenergic blocking therapy has been attributed to cardiac effects of these drugs rather than to effects on the blood vessels (4, 5, 8, 11). The correlation between the reduction in heart rate and systolic blood pressure during exercise in hypertensive patients during Aptin treatment gives further support for such an interpretation. In acute tests with Aptin as well as with propranolol, Johnsson (2) found that a decrease in mean arterial blood pressure of about 10 mm Hg during beta blockade was accompanied by a decrease in cardiac output in some hypertensive patients.

Furberg (1) has shown that the effect of beta blockade of the heart rate during exercise expressed as W_{170} was generally most pronounced in subjects with signs of a hyperkinetic circulation and least in those with signs of a hypokinetic circulation. Sannerstedt (7) found a more hyperkinetic circulation in hypertensive patients without evidence of organic changes in the cardiovascular system than in patients with evidence of organic damage. Judging from these investigations an antihypertensive effect of Aptin would be expected primarily in patients with signs of a hyperkinetic circulation and without evidence of organic cardiac damage.

The antihypertensive effect of Aptin in patients with arterial hypertension may be regarded as moderate. It is possible that beta adrenergic blocking therapy has a therapeutic value in combination with other antihypertensive drugs.

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ANTIHYPERTENSIVE THERAPY WITH ALPRENOLOL A β ADRENERGIC RECEPTOR ANTAGONIST

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Abstract In a double-blind cross-over study on 11 patients with hypertension the therapeutic effect of the β adrenergic receptor antagonist alprenolol has been compared with that of placebo each preparation being administered for 12 weeks. Alprenolol was administered in daily doses of 80 mg during the first two weeks, 300 mg during the following two weeks and 400 mg for the remaining eight weeks. Compared to placebo alprenolol produced on the average statistically significant reductions of about 70 mm Hg systolic and 10 mm Hg diastolic blood pressure in supine as well as in standing and sitting positions. The hypotensive effect of alprenolol followed two different time courses. In one group of patients the effect was fully developed after the first two weeks of treatment. In another group the effect continued to increase throughout the twelve week treatment period reaching an average reduction of 36 mm Hg systolic and 13 mm Hg diastolic pressure. At the beginning of treatment with the higher dose of alprenolol one patient complained of attacks of effort angina probably related to a pronounced reduction of the blood pressure. Otherwise no side-effects or complications were observed.

A reduction of the arterial blood pressure has been observed in hypertensive patients treated with β adrenergic receptor blocking agents such as pronethalol (21, 26), propranolol (13, 20, 22, 23, 31), and oxprenolol (6, 7). In most studies the β blockers were given in moderate dosage and found to have an antihypertensive effectiveness of about the same order of magnitude as that of the thiazide diuretics (7, 13, 20). However, in the most extensive and long lasting studies reported Prichard and Gillam (22) found propranolol given in individualized and often extremely high doses to be a very active antihypertensive agent of at least the same potency as betanidine, guanethidine and methyl dop. It is generally agreed that the β blockers do not pro-

duce postural or exertional hypotension (22). There are controversial reports as to the time curve for the antihypertensive effect of these agents. The antihypertensive action of propranolol was found to be characterized by a gradual onset over several weeks in one study (22). Others found that the effect was fully established already after the first day of treatment (31).

The present study concerns an evaluation of the antihypertensive action of alprenolol (Baptin[®], Aptin[®]), a β receptor antagonist for which therapeutic effectiveness has been demonstrated in angina pectoris (4, 5), cardiac arrhythmias (16) and neurocirculatory asthenia (19). Pharmacological studies in animals indicate that alprenolol differs from propranolol by having a moderate β stimulating activity (32) and this property modifies the haemodynamic effects of the drug in both animals and man (11).

The antihypertensive effect of alprenolol has been investigated on outpatients with arterial hypertension in two parallel double blind studies running for seven months. In one study (14) the effect of alprenolol was examined on patients at rest as well as during exercise while in the present study the effect of the drug was studied on patients at rest only.

MATERIAL

The trial began with 14 patients having supine systolic pressures not less than 150 mm Hg and diastolic pressures not less than 100 mm Hg. During the pretreatment period blood pressure values below these limits were recorded in three of the patients and they were subsequently excluded from the study. The 11 remaining patients had pretreatment supine systolic pressure between 155 and 200 mm Hg and diastolic pres-

Table I Clinical information concerning investigated patients

The symbol + for hypertensive eye ground changes indicates attenuated arterioles or calibre-changing arterioles

Pat	Age	Sex	Hypertensive eye ground changes	Heart volume ml BSA	Left ventricular hypertrophy on ECG
C	31	♀	+	340	0
K	58	♂	+	470	(+)
J	61	♂	+	500	0
A	51	♂	+	410	0
B	56	♂	+	410	+
AA	46	♂	+	460	0
M	57	♂	+	500	+
F	30	♀	+	260	0
N	43	♂	+	—	0
L	56	♂	0	420	0
O	50	♂	+	350	0

ures between 100 and 130 mm Hg. Patients with bronchial asthma, chronic bronchitis, heart failure grade III to IV, retinopathy or renal hypertension were not included in the trial.

A summary of the clinical information concerning the 11 patients taking part in the study is presented in Table I.

METHOD

The trial was carried out on a double-blind cross-over design. After an initial control period of two weeks without treatment, all patients received placebo for a further two weeks. Thereafter followed two 12 week treatment periods with placebo and alprenolol respectively in randomized order. Alprenolol was given in a dose of 100 mg four times daily after a run-in period of four weeks, the daily dose being 200 mg for the first two weeks and 300 mg for the following two weeks. The

placebo tablets were identical in taste and appearance with alprenolol tablets.

The patients attended the clinic every second week and on each visit their blood pressure was recorded with a cuff in sitting, standing and supine positions after 5 min talking. Side-effects were assessed by a symptom questionnaire which was read in the patient's presence at the start of the trial and at the end of each 12 week period of treatment. Blood and urine estimations were carried out to detect any possible toxic effect of the drug. The following variables were studied: haemoglobin, white blood corpuscle count, reticulocytes, thrombocytes, SGOT, SGPT, alkaline phosphatases, bilirubin, creatinine, serum electrolytes, Albitux, Clinistix and Coombs test.

For statistical evaluation of the results presented in Table II, the average systolic and diastolic blood pressure levels measured at the end of the 10th and 11th week of alprenolol treatment were compared with those recorded after the corresponding periods of placebo treatment. Patient F pursued the placebo treatment for only 6 weeks (see Results) and the last blood pressure noted in the placebo period was taken to represent the placebo value in the statistical evaluation.

Results are reported as means \pm standard errors of the mean. The statistical analysis was based on the *t* test (10).

RESULTS

The arterial blood pressure was lower during the alprenolol period than during the placebo period in ten of the eleven patients. As shown in Table II, there was after 10–12 weeks of alprenolol treatment about the same average blood pressure reduction in supine as in sitting and standing positions, the systolic pressure being decreased by about 20 mm Hg and the diastolic pressure by about 10 mm Hg.

Figs 1 and 2 show the average supine and standing blood pressures recorded in each period.

Table II Average blood pressures recorded before and 10–12 weeks after treatment with alprenolol or placebo in the 11 patients

	Initial control	After 10–12 weeks on alprenolol	After 10–12 weeks on placebo	Differences alprenolol placebo
<i>Supine</i>				
Systolic	173 \pm 4.7	153 \pm 4.7	176 \pm 2.9	-23 \pm 4.9 ($p < 0.001$)
Diastolic	111 \pm 2.6	101 \pm 3.0	110 \pm 3.2	-9 \pm 3.1 ($p < 0.07$)
<i>Standing</i>				
Systolic	173 \pm 5.1	152 \pm 3.9	171 \pm 4.1	-19 \pm 4.9 ($p < 0.01$)
Diastolic	121 \pm 2.3	108 \pm 2.1	121 \pm 2.8	-13 \pm 3.3 ($p < 0.01$)
<i>Sitting</i>				
Systolic	179 \pm 4.9	153 \pm 4.9	174 \pm 4.4	-21 \pm 6.1 ($p < 0.01$)
Diastolic	115 \pm 3.0	103 \pm 2.5	114 \pm 3.2	-11 \pm 3.9 ($p < 0.07$)

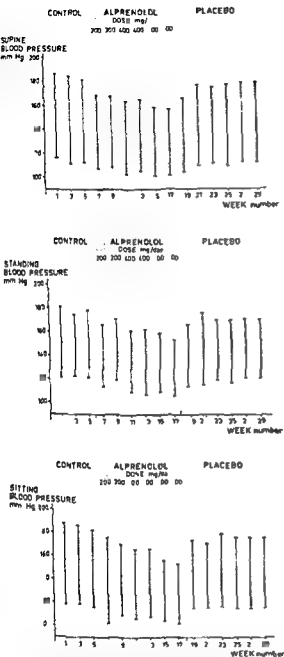


Fig 1 Average blood pressures recorded in the seven patients in whom the alprenolol period preceded the placebo period

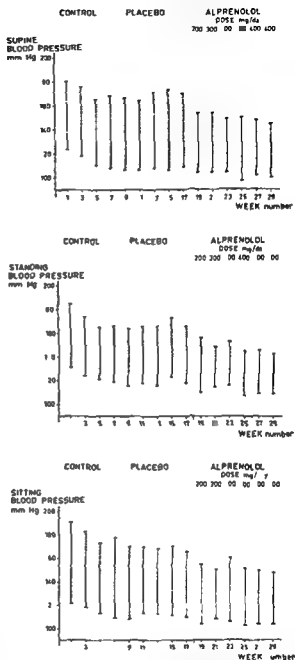


Fig 2 Average blood pressures recorded in the four patients in whom the placebo period preceded the alprenolol period

It can be seen that the blood pressure was reduced after two weeks' treatment with 200 mg alprenolol daily and there was some further reduction during the following ten weeks of therapy. When alprenolol was withdrawn the blood pressure

returned to pre-treatment control values within four weeks (Fig 1).

The individual time-effect curves during alprenolol treatment followed two different patterns. In six patients (Fig 3) there was some decrease

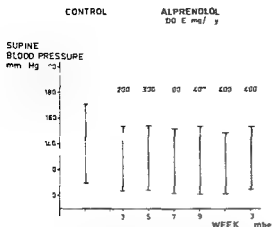


Fig 3 Average supine blood pressures before and during alprenolol therapy in six patients whose response to the drug was fully developed after two weeks treatment.

of the blood pressure after the first two weeks treatment with 200 mg alprenolol (mean reduction of supine systolic pressure 17 ± 5 mm Hg ($p < 0.05$) and of diastolic pressure 6 ± 3 mm Hg) and the blood pressure showed little further reduction during the following ten weeks with administration of higher doses. In the remaining four patients who responded to the drug (Fig 4) the supine blood pressure was also somewhat reduced after the first two weeks of treatment but the blood pressure then continued to decrease throughout the following ten weeks of alprenolol therapy. From the second to the last four weeks

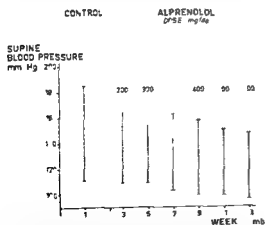


Fig 4 Average supine blood pressures before and during alprenolol therapy in four patients whose response to the drug developed gradually during the 12 weeks treatment.

period of alprenolol treatment the supine systolic blood pressure was in this group reduced by 12 ± 3 mm Hg ($p < 0.05$) and the diastolic pressure by 6 ± 1 mm Hg ($p < 0.05$). At the end of the 12 week alprenolol period the mean supine systolic and diastolic blood pressures of these patients were reduced by 36 ± 7 mm Hg ($p < 0.05$) and 23 ± 5 mm Hg ($p < 0.05$) respectively if the blood pressures recorded at the corresponding placebo period are taken as reference values.

In the two groups of patients the standing and sitting blood pressures during alprenolol treatment followed on the whole the same differentiated time patterns as characterized the supine blood pressure. The blood pressure variations during placebo treatment showed no striking differences between the two groups. The patients with a gradual hypotensive response had on an average a somewhat higher control systolic blood pressure than the other group with a purely acute response but the difference (15 ± 8 mm Hg) was not statistically significant. There was no obvious difference as regards age, sex and severity of hypertensive disease between the two groups of patients.

Two patients who showed a marked response to alprenolol deserve some comments. Case F, a 30 year-old woman, was divorced and had several family problems. She had typical symptoms of neurocirculatory asthenia, i.e. palpitations, sighing type of dyspnoea and uncharacteristic pain localized to the region of the apex of the heart. This patient had a supine control blood pressure of about 200/125. Under alprenolol therapy the pressure showed a gradual fall to 140/100 at the end of the 12 week period (Fig 5). After withdrawal of alprenolol and introduction of placebo the blood pressure increased rapidly to pretreatment control levels. The patient's treatment course was interrupted after six weeks placebo treatment and she was withdrawn from the rest of the study.

Patient L was a 56 year-old man without previous signs or symptoms of coronary heart disease. This patient responded promptly to two weeks therapy with 200 mg alprenolol daily, his supine blood pressure being reduced from a control level of 180/105 mm Hg to 140/90 mm Hg. The blood pressure remained at this level on treatment with 400 mg alprenolol except that the low value of 120/90 mm Hg was recorded after the

first two weeks on this dosage. During these two weeks the patient complained of almost daily attacks of angina pectoris on exercise which however disappeared in the next period when the blood pressure apparently had stabilized at a slightly higher level.

With the exception of the appearance of angina pectoris in patient L, no side-effects or complications were observed. The laboratory investigations showed no significant changes in hepatic or renal function, serum electrolytes or haematological status in any patient.

DISCUSSION

The results with alprenolol in the present study correspond closely to those obtained in the parallel study by Furberg and Michaelson (14). The antihypertensive effect observed under alprenolol treatment is of the same order of magnitude as that reported for moderate doses of propranolol in several studies (13, 20, 31).

There are several reasons to assume that in the dosage most commonly used the antihypertensive effects of alprenolol and propranolol can be ascribed to their β receptor blocking action. Haemodynamic studies carried out on hypertensive patients at rest indicate that the antihypertensive actions of alprenolol (3) and propranolol (3, 13, 27) are due to a reduction of cardiac output. The antihypertensive effects of propranolol and alprenolol during physical exercise have also been found to be due to a reduction of cardiac output (3, 28). In alprenolol-treated patients subjected to standardized exercise, Furberg and Michaelson (14) found a correlation between the decrease in the systolic blood pressure and the decrease in heart rate. As could be expected for β blocking agents, both alprenolol and propranolol have been found to produce greater absolute blood pressure reductions in situations of increased endogenous β sympathetic activity such as exercise (14, 28) than they elicit under resting conditions. On the other hand, the agents do not produce such a marked hypotensive response to exercise as that which characterizes adrenergic neurone blocking agents (cf. 22). Both alprenolol and propranolol have consistently been found to lower supine and standing blood pressures to about the same degree. The lack of postural and exertional hypotension is to be ex-

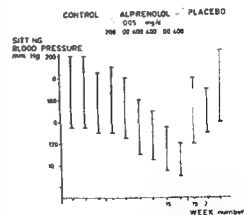
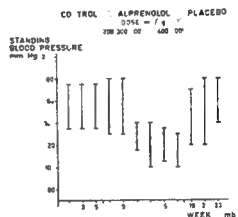
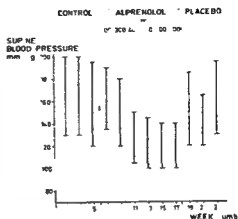


Fig. 5. The effect of alprenolol treatment in a patient with hypertension and symptoms of neurocirculatory asthenia.

pected since the homeostatic reflex control of the peripheral resistance and capacitance vessels is mediated via adrenergic receptors in vascular

smooth muscle (18). A single oral dose of 100 mg alprenolol elicits a marked cardiovascular β blockade approximately corresponding to that produced by 50 mg propranolol (32). Hitherto reported prolonged therapeutic studies with alprenolol and propranolol in hypertension indicate that there may be a similar 2:1 relationship between equipotent antihypertensive doses of the two drugs (13, 14, 20, 31). In very high doses both alprenolol and propranolol have in addition to β blockade a direct depressant action on cardiovascular contractility (32). This mechanism might have been of contributory importance for the hypotensive effects reported by Prichard and Gillam (22) as considerably larger doses of propranolol up to 4 g daily were given to many of their patients.

According to older concepts the hypertensive patient is haemodynamically characterized by an increased peripheral vascular resistance with normal or slightly reduced cardiac output (12). This seems still to be valid for most patients with severe hypertension and those with hypertension of long duration but more recent investigations indicate a different haemodynamic situation in many patients with mild or moderate hypertension who have an increased cardiac output with normal peripheral vascular resistance (1, 2, 9, 15, 17, 25, 30). There is some evidence that patients with high output hypertension develop high resistance hypertension as the hypertensive disease progresses (2, 8). After intravenous administration of propranolol Bello et al (2) and Kuramoto et al (15) found a more marked reduction of cardiac output in patients with high output hypertension than in those with low output hypertension. This finding indicates that the cardiac output in the former group of patients is at least to a substantial degree controlled by sympathetic tone. Altogether the studies on the haemodynamic situation in hypertension discussed above make β receptor blockade an attractive therapeutic principle for patients in earlier stages of the hypertensive disease.

In the present series the duration and the haemodynamic characteristics of the hypertension were unknown. It is of interest however that of the two patients showing the most marked hypotensive response to alprenolol one was the only patient with clinical signs and symptoms of hyperkinetic circulation and the other was the only

patient devoid of hypertensive eye ground changes. Otherwise in this small series there was no obvious connection between the magnitude of the hypotensive effect on the one hand and the age of the patients and the severity of their hypertensive disease on the other.

In the present study the patients could be divided into two groups in terms of the time curve for the hypotensive response to alprenolol. In one group the full effect of the drug was developed after two weeks treatment while in the other group the effect continued to increase throughout the 12 week treatment period. This finding gains more significance by the fact that the same differentiation could be made on re-examination of the data obtained in the parallel study of Furberg and Michaelsson (14). The present data give no indication as to the cause of these different time effect curves. From both practical and theoretical points of view it is of interest that in about one third of the patients in the present study alprenolol elicited a rather marked antihypertensive effect characterized by a gradual onset over several months. Prichard and Gillam (22) have observed a similar gradual onset of the hypotensive response to propranolol. These authors have suggested that as the cardiac β blocking effect of propranolol appears to be fully developed immediately after commencing the treatment the gradual hypotensive effect is due to a slow reconditioning of the baroreceptors. The studies of Frohlich et al (13) indicate that the antihypertensive effect observed after prolonged treatment with propranolol is still due to a reduced cardiac output.

In the present study and in that of Furberg and Michaelsson (14) all but one patient tolerated a daily dose of 400 mg alprenolol without any complications or side-effects. One patient in the present study reacted to this dose of alprenolol by a pronounced reduction of the blood pressure and complaints of exertional angina pectoris. As this patient reached normotension on a daily dose of 200 mg alprenolol the appearance of angina pectoris after the higher dose could probably be regarded as a response to a supraoptimal therapeutic dose of the drug leading to a drastic reduction of the coronary perfusion pressure. The reaction of this patient may indicate that when used in angina pectoris treatment an overdose of alprenolol may in certain cases produce a "para-

doxical angina due to excessive hypotension as has also been described for nitroglycerine (24). The case reported above illustrates the importance of starting alprenolol therapy with lower dosage and of individualizing the maintenance dose. Prichard and Gillam (22) have recently emphasized the importance of individualizing the dose of propranolol in antihypertensive treatment and report that a well tolerated normotension could be maintained on daily doses varying from 10 mg to 4000 mg. In view of these data it seems well worth trying higher doses of alprenolol in patients who do not reach normotension on daily doses of 400 mg or below.

Alprenolol and propranolol appear to reduce blood pressure to about the same degree but with different haemodynamic effect patterns. Both after acute (2, 29) and chronic (13) administration of propranolol to hypertensive patients the drug induced reduction of the cardiac output was as a rule accompanied by an increase of the peripheral vascular resistance. This type of response to propranolol was also found by Bergman et al (3) who compared the acute haemodynamic effects of this compound and alprenolol in hypertensive patients in supine position. Alprenolol reduced the blood pressure to the same extent as propranolol but *did not change* the peripheral vascular resistance. The effects of propranolol were probably a consequence of β receptor blockade in the heart and peripheral blood vessels whereas the effects of alprenolol were presumably a result of β receptor blockade combined with a small β receptor stimulation in the heart and blood vessels (11).

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IMMUNOGLOBULINS IN SERUM AND JEJUNAL BIOPSIES IN NON TROPICAL SPRUE

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Abstract A study has been made of 12 patients with non tropical sprue. Immunochemical determinations of serum IgG, IgA and IgM were carried out prior to gluten free diet (9 patients) and during treatment (10 patients). Immunofluorescent microscopy of jejunal biopsies from the same patients was performed prior to (10 cases) and during treatment (6 cases). Fluorescent antisera specifically reacting with IgG, IgA and IgM were applied and the cells containing these immunoglobulins were differentiated and quantitated accordingly.

Serum IgM which was decreased in untreated patients was normalized during treatment. Serum levels of IgG and IgA were normal in both treated and untreated cases of sprue. Increased numbers of cells containing IgG and IgM were found. The immunoglobulin levels of serum were not correlated to the area of cells containing the corresponding immunoglobulins.

The human intestinal mucosa is known to contain a large number of plasma cells. In immune histochemical studies it has been shown that the majority of these mucosal plasma cells contain IgA immunoglobulin (1, 11) whereas cells containing IgM or IgG immunoglobulin are less numerous.

Considering the very limited number of cells containing IgA immunoglobulin in lymph nodes, bone marrow and spleen (8) it appears that a large proportion of the total body content of IgA containing cells is confined to the intestinal mucosa. A correlation between the serum level of IgA and the number of intestinal plasma cells containing this immunoglobulin has been found in patients with marked hypogammaglobulinaemia (2, 4, 7). In sprue and Crohn's disease a similar parallelism between a moderately elevated serum IgA and intestinal content of IgA

cells has been found (4, 10). However, in the latter studies the determination of the intestinal content of IgA-cells was only semiquantitative. In a recent report a deficiency in serum IgM was found in patients with untreated sprue. During treatment with gluten free diet serum IgM was normalized (5).

The present study was undertaken to provide information about the correlation between the quantitatively determined intestinal amount of immunoglobulin containing cells and the serum immunoglobulin level in controls and in patients with non tropical sprue. In addition the effect of gluten free diet on these parameters was studied.

MATERIAL

Twelve patients aged 18 to 60 years with gluten induced enteropathy. The diagnosis was based on clinical and biochemical evidence of malabsorption and subtotal or total atrophy of the proximal jejunal mucosa. In all cases a clinical and biochemical improvement had taken place after a gluten free diet. Table 1 summarizes the findings in each patient. Intestinal biopsy was performed in all cases to establish the diagnosis.

Immunofluorescent studies were carried out prior to treatment in ten cases and in six cases after 8-36 months on a gluten free diet.

Serum immunoglobulins were determined before dietary treatment in nine cases and after dietary treatment in ten cases. Blood samples and intestinal biopsy specimens were taken on the same day.

METHODS

The concentration of IgG, IgA and IgM in sera stored at -18° for 2 days-1 year was estimated by electrophoresis on antibody containing agarose (14). As refer

Table I

No	Sex	Age	Treatment	Duration of symptoms/ diet years	Faecal fat excretion	D xylose ^a absorption	Jejunal pathology	Immuno-fluorescent study per formed	Serum immuno-globulin level determined
1	♂	61	No	4	48	2.1	Severe	No	No
			+	2	21	6.1	Severe	+	+
2	o	58	No	2	75	1.1	Severe	+	+
			+	9/12				No	+
3	♂	17	No	7	23	1.5	Severe	+	+
			+	3/12				No	+
4	o	50	No	8	5	8.0	Severe	No	No
			+	3	6	11.0	Moderate	+	+
5		44	No	30	33	5.1	Severe	+	+
			+	1	4	4.4	Severe	+	+
6	-	24	No	3	8	3.1	Severe	+	+
			+	9/12	2	7.9	Severe	+	+
7		26	No	2	15	3.2	Severe	+	+
			+	8/12	2			No	+
8		17	No	10	83	1.4	Severe	+	+
			+	9/12	18	Normal ^b	Severe	+	+
9	■	27	No	1	70	3.3	Severe	+	No
			+	6/12				No	+
10	o	54	No	30	30	4.2	Severe	+	+
11	o	18	No	6	17	4.6	Moderate	+	+
			+	8/12	2	7.5	Slight	+	+
12		47	No	19	22	2.9	Severe	+	+

^a Grams per day from a 3-day stool collection. Normal is less than 7 g.

^b Grams excretion in 4-hour urine collection after ingestion of 75 g. Normal greater than 50 g.

Determined after blood values

in the quantitation Human Standard Serum Op no 166 (Behringwerke Marburg, Lahn Germany) was used. The rabbit antibodies used were antihuman IgG (Dakopatts Ltd Copenhagen) antihuman IgA and antihuman IgM (Behringwerke Marburg, Lahn Germany). The serum level of IgG, IgA and IgM was determined in 44 healthy members of the medical staff (average age 33 years) and 45 blood donors (average age 39 years). The distribution of the immunoglobulin values showed a skewed distribution. In all the calculations therefore log values were used. The 95% range for IgG was 7.2–15.1 g/l (mean 10.4 g/l) for IgA 0.74–3.06 g/l (mean 1.50 g/l) and for IgM 0.23–1.33 g/l (mean 0.56 g/l).

Biopsy specimen material

The jejunal biopsies were obtained with a peroral hydraulic multiple biopsy tube. All specimens were taken 10–30 cm distal of Treitz ligament and were immediately frozen and stored at -70°C.

Immunofluorescent methods

The details of the procedure used for preparation of fluorescein-isothiocyanate conjugates as well as the technique of labelling with the fluorescent antisera, microscopic measurements and control of specificity have been fully described in a previous publication (12) and need only be briefly summarized here.

Antisera against human IgA, IgM and IgG were obtained from a commercial laboratory. A crude globulin fraction was precipitated by 17 M ammoniumsulphate and was conjugated with fluorescein isothiocyanate at an alkaline pH. By chromatography on a Sephadex G 25 column the free fluorescein isothiocyanate was removed from the antisera. Each conjugate was absorbed with guinea pig liver powder. Tissue sections 4–6 µm thick were air-dried and fixed for 3 minutes in absolute methanol and preincubated with one drop of unconjugated anti IgA, IgM or IgG sera (of the same batch as used for preparation of fluorescein conjugates). After washing in Coon's buffer the sections were incubated with fluorescent anti IgA, IgM or IgG.

A Reichert Zetopan microscope equipped with a photomicrotome was used for fluorescence microscopy. Dark field technique was employed. Photomicrographs were obtained under standard magnification with Anscochrome 24 DIN daylight film. The photomicrographs were projected on a white sheet of paper. The areas to be measured were drawn by hand cut out and weighed. As the immunoglobulin-containing cells were confined to the interstitial tissue they were quantitated by relating the fluorescent area to the total area of the interstitial tissue in a given visual field. Of each section used for quantitation six visual fields were examined and the mean of these determinations was used.

In eight controls aged 71 to 60 years mean values (\pm S.D.) of immunoglobulin-containing cells as % of

interstitial area were calculated. The following values were found IgA 144 \pm 69 IgM 58 \pm 35 (IG 0.3 \pm 0.2%)

RESULTS

Serum Concentration of IgG IgA and IgM

The concentration of IgG IgA and IgM in serum before and during dietary treatment is shown in Fig 1

IgG

The mean serum IgG was 9.0 g/l (95% range 7.8–20.5 g/l) before treatment. The mean serum IgG was 11.9 g/l (95% range 7.4–19.2 g/l) during treatment which is not significantly different from the normal mean ($p > 0.3$)

In seven patients the mean serum IgG before and during treatment changed from 8.4 to 10.7 g/l. This alteration was not significant ($p > 0.05$)

IgA

The mean serum IgA was 1.56 g/l (95% range 0.27–7.28 g/l) before treatment and 1.62 g/l (95% range 0.52–6.41 g/l) during treatment which is not significantly different from normal ($p > 0.8$). In seven patients serum IgA was followed before and during treatment but the alteration in mean serum IgA from 1.39 g/l to 1.36 g/l is not significant ($p > 0.8$)

IgM

The mean serum IgM before treatment was 0.30 g/l (95% range 0.12–1.04 g/l) which was significantly lower than normal mean ($p < 0.001$). During treatment the mean IgM was 0.56 g/l (95% range 0.34–1.06 g/l) which is not different from the normal mean ($p > 0.9$). The mean serum IgM rose from 0.29 to 0.54 g/l in seven patients in whom it was determined before and during treatment. This alteration is significant ($p < 0.005$)

Cells Containing IgG IgA and IgM in Jejunal Biopsies

IgG

The area of IgG cells as % of interstitial area was 9.9 (s.d. 7.3%) before treatment during treatment 5.3% (s.d. 2.3%) which in both cases are significantly higher than in controls ($p < 0.001$). In four patients the area of IgG cells was determined before and during treatment. In these four patients a slight fall in IgG cells was seen

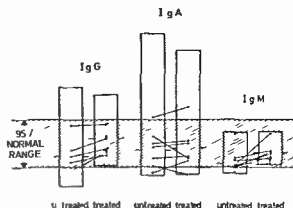


Fig 1 Concentration of IgG IgA and IgM in serum before and during dietary treatment in non tropical sprue 95% range is indicated

from 7.5% to 6.0% but this was not significant ($p > 0.3$)

IgA

The area of IgA cells in patients with untreated sprue was 18.0 (s.d. 9.7%) during gluten free diet 12.5 (s.d. 2.5%) which are not different from normal values ($p > 0.1$). In four patients the area of IgA cells was 15.3% before and 11.9% after start of diet. This alteration is not significant ($p > 0.1$)

IgM

In untreated sprue the area of IgM cells was 12.1 (s.d. 2.4%) during treatment 9.9% (s.d. 3.6%) which are significantly higher than in controls ($p < 0.001$ and $p < 0.025$ respectively). In four patients the area of IgM cells was determined before and during treatment and a slight fall was seen from 12.7% to 10.3% but this is not significant ($p > 0.2$)

Correlation Between Immunoglobulin containing Cells in Jejunum and Serum Immunoglobulin Levels

Controls

In seven controls both serum values and jejunal area of plasma cells of the three immunoglobulins were determined. The values are shown in Fig 2. No correlation between the serum level and the jejunal area of cells containing the same immunoglobulin could be found for any of the three immunoglobulins ($p > 0.1$)

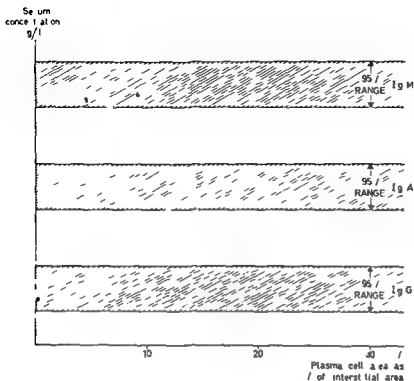


Fig 2 Correlation between the serum level of IgG, IgA and IgM and the area of jejunal cell containing the same immunoglobulins in seven controls

Sprue patients

Using serum values and jejunal interstitial plasma cell areas for the three immunoglobulins shown in Fig 3. Values for both treated and untreated patients are included. No correlation between the serum level and the jejunal plasma cell area could be found for any of the three

immunoglobulins in untreated or treated patients ($p > 0.1$)

DISCUSSION

Serum IgM was significantly decreased in untreated sprue patients. During dietary treatment

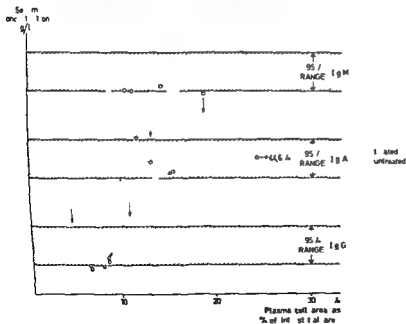


Fig 3 Correlation between the serum level of IgG, IgA, and IgM and the area of jejunal cells containing the same immunoglobulins in patients with untreated and treated sprue. Δ treated \circ untreated

serum IgM was normalized. This is in agreement with the results published by Hobbs and Hepner (5) who found a similar low serum IgM which normalized during treatment. Serum IgA showed no alterations compared to normals either before or during treatment. This was observed too by Hobbs and Hepner (5) but is in contrast to the report of Eidelman et al (3) and Poen et al (10) who found elevated values of serum IgA. Serum IgG was normal both in treated and untreated patients.

The low serum IgM level might be due to gastrointestinal protein loss which is known to take place in untreated non tropical sprue (6, 9, 13). However a gastrointestinal loss cannot explain the discrepancy between the decreased serum IgM and the normal serum IgA and IgG and a selective loss of the high molecular IgM is unlikely. A decreased IgM synthesis seems not very likely as the immunofluorescence microcopy showed a significantly elevated number of IgM containing cells in the jejunal mucosa.

Further elucidation of these problems must await protein turnover studies.

The results of the immunofluorescent studies in non tropical sprue have been published in part elsewhere (12). As in normals the IgA cells are most numerous in the jejunal mucosa but a significant increase in IgM and IgG containing cells was found in both groups of sprue patients and the total number of immunoglobulin containing cells was increased. In an immunofluorescence study of intestinal biopsies from sprue patients Rubin et al (11) found a similar increase of the immunoglobulin containing cells but noticed a larger predominance of IgA cells than found in this study. This difference might be due to the composition of the case material as the jejunal pathology in the present study appeared to be more pronounced than in the material of Rubin et al.

In patient number 3 who had normal serum concentration of immunoglobulins an extreme elevation of the total number of immunoglobulin containing cells in the jejunal mucosa was due to an increase in the IgG and IgA cells. The patient is under investigation whether the infantilism is primary or secondary.

No correlation was found between corresponding values of the serum concentration and the area of immunoglobulin-containing cells in je-

junum for any of the immunoglobulins studied. As a considerable part of the IgA containing cells of the body are probably found in the intestinal wall a positive correlation could be expected as far as IgA is concerned.

In sprue patients in whom both elevated serum IgA and jejunal area of IgA cells have been described (3, 10) the proper condition for demonstrating this correlation should be present. In fact we did not find any alterations of IgA in serum and only a slight but insignificant increase of IgA cells in jejunum and with these minimal changes no correlation could be demonstrated.

ACKNOWLEDGEMENTS

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Addendum. After completion of the study it appeared that the anti IgA preparation employed had also some specific activity against secretory piece since colostrum had been used for immunisation. Thus a new anti IgA preparation was procured. This preparation was tested in serial dilution against normal human serum, light chains, myeloma and Waldenström sera and against colostrum. It proved to possess specific reactivity only against serum IgA. Seven of the biopsies (3 controls, 4 patients with non tropical sprue) included in this investigation were re-examined with the new and the old anti IgA preparation.

Quantitation studies showed that there was no difference in the number of interstitial cells giving specific fluorescence with the two antisera. The faint staining of the epithelial cells induced by the anti IgA with activity against secretory piece was absent with the new anti IgA preparation and consequently this staining cannot be considered unspecific. Studies with specific antisera against secretory piece and serum IgA are now being undertaken.

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STUDIES OF PITUITARY ADRENOCORTICAL FUNCTION IN ULCERATIVE COLITIS

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Abstract The pituitary adrenal function has been evaluated in 41 patients with well defined ulcerative colitis. No dysfunction was shown. In three cases a false negative response in urinary 17 KGS was found during the metyrapone test.

The variable course of ulcerative colitis and the beneficial effect of glucocorticoids in some cases may suggest a relative insufficiency of the pituitary adrenal system as a causal factor in exacerbations of the disease. This could parallel findings in bronchial asthma in which Robson and Kulborn (12) found a diminished response of the adrenal cortex to exogenous corticotrophin in 21 out of 34 patients with continuous asthma.

The purpose of the present study was to evaluate the pituitary adrenal function in a group of patients with well defined ulcerative colitis. It was done by means of the plasma concentration of 11 hydroxy-corticosteroids (11 OHCS) and the urinary excretion of 17 ketogenic steroids (17 KGS) spontaneously and during the administration of corticotrophin and metyrapone.

MATERIAL AND METHODS

The material comprised 41 patients with ulcerative colitis consecutively admitted to the Gastroenterological Department B. The age of the patients varied from 16 to 75 years. The distribution with respect to age and sex was not different from that found in a larger group of patients with ulcerative colitis (4). The diagnosis of ulcerative colitis was based upon the fulfilment of at least two of the following four sets of diagnostic criteria:

1. A case history including bloody diarrhoea and/or passage of blood between the proper bowel movements. The presence of diarrhoea and/or rectal tenesmus is not necessary.

2. Sigmoidoscopic findings (at least two of the following findings must be present): (a) granulated mucosa (b) increased friability (c) haemorrhagic and/or purulent contents in bowel lumen (d) ulcerations punctate or larger (e) pseudopolyps.

3. Cytological and/or biopsical signs of inflammation. Cytology: presence of inflammatory leucocytes (presence of eosinophils not necessary). Biopsy: presence of inflammatory leucocytes in the mucosal and submucosal layers.

4. Radiological finding of (a) spicula (b) small or large ulcers (c) pseudopolyps. (In patients presenting colonic diverticula three instead of two major diagnostic criteria headings 1-4 to be fulfilled for the diagnosis of ulcerative colitis.)

None of the patients had been treated with glucocorticoids within one year prior to the study and 39 of the patients had never been treated with glucocorticoids.

The excretion of 17 KGS was determined in 24-hour urinary specimens by the method of Jørgensen (10) at the Hormone Department of Statens Serum Institut Copenhagen.

The plasma concentration of 11-OHCS was determined using the fluorimetric technique of DeMoore et al (8) as described by Nielsen and Asfeldt (11). For determination of cortisol and 11-deoxycortisol a double isotope technique was used (3, 6). The latter two analyses were carried out by Medicinsk Laboratorium Copenhagen.

All the patients were admitted to hospital during the study. None of them were given sedatives or narcotics. After a two-day control period the ACTH test was carried out. If the concentration of 11-OHCS in plasma was initially normal and had doubled or more 4 hours after the intramuscular administration of 30 IU corticotrophin (Acton prolongatum®) the test was considered positive (1). If this was not the case the administration of corticotrophin was continued 6-hourly for 3 days. A doubling of the daily excretion of 17 KGS was considered an adequate response from the adrenal cortex (9).

After a 3-day interval the metyrapone test was carried out starting with a new 24-hour urine collection. Then metyrapone was given hourly in 500 mg doses for 24 hours beginning at noon. A blood sample for the specific determination of cortisol and 11-deoxycortisol was drawn

THE SERUM IMMUNOGLOBULIN AND β 1C/ β 1A GLOBULIN LEVELS IN RHEUMATOID ARTHRITIS

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Abstract The immunoglobulins IgG, IgA and IgM and the β 1A globulin have been determined by the single radiation immunodiffusion technique in serum samples from 55 RA patients and 48 healthy controls. The average total Ig level and the IgG level were higher in RA sera. The IgA and IgM levels were also somewhat higher but the difference was not statistically significant if the age group was taken into consideration. The β 1A globulin content was similar in the RA sera and the controls. This globulin was positively correlated to the total Ig and to the IgG level in the RA series and inversely to the IgM level in the normal series. The IgG level rose with increasing age both in the RA series and in the whole series. In the normal series the IgM level diminished with increasing age.

The auto-immune nature of rheumatoid arthritis (RA) has not been unequivocally established even if it has been suspected for a long time (1-4, 12). The rheumatoid factor (RF) is an immunoglobulin of the IgM class reacting with human or animal IgG (1, 11, 12). For diagnostic purposes RF is demonstrated in the serum by the Waaler-Rose test or similar procedures but the factor has also been found in the tissues of patients suffering from RA (1, 8, 10, 15). RA might thus be characterized as a disease accompanied by noteworthy immunological phenomena even if the exact pathogenetic role of RF is still unknown. These phenomena are mainly humoral and changes are to be expected in the levels of circulating immunoglobulins (Ig) and complement (C).

In active RA the total Ig level is usually high (2, 3, 21, 23) and raised values of IgG were found by Muller and Muller-von Voigt (16) in a small material. Mackiewicz and Fenrych (13) found elevated IgM values in most cases of RA. Svartz (19) on the other hand found very high

values in some cases but most of her RA patients had normal amounts of IgM in their serum. By the application of a titration method on agar plates we (25) have previously found larger amounts of IgM in the serum of RA patients than in that of other hospital patients and of healthy blood donors. Veys and Claessens (24) found normal IgM levels in RA according to them the age factor is very important and Ig levels should be compared only in corresponding age groups. Within the age groups the average levels of IgG, IgM and IgA in serum samples from patients with RA did not differ significantly from normal sera although in RF positive sera the levels of all three Ig classes tended to be raised.

The total C activity of serum from patients with RA has been studied by several groups of research workers and values which are normal to somewhat elevated have been recorded in most cases (3, 7, 19, 26). However Gross et al (5) found low values in 10 of 24 cases and Boni (3) established that the values are low in malignant forms of the disease. Hanauer and Christian (6) determined both the total C and C1 and found normal or elevated values in four cases but low values in a patient seriously ill with arteritis. Petkin and Zvaifler (18) noted diminished C activity in the synovial fluid of RA patients and according to Hedberg (7) it is negatively correlated to the serum RF titre.

Quantitatively β 1C globulin is the most important fraction of C (20). It belongs to C3 and is in vitro converted to β 1A globulin which can be determined by precipitation in agar gel (10, 17, 19). A close correlation has been found between this globulin and the total C' activity (17).

Table 1 The average levels of immunoglobulins and β 1 A globulin in RA sera and in normal sera

Type of protein	Series	No of observations	Mean (mg/100 ml)	S.D.	p-value
Total Ig	RA	51	2172	815	<0.0005
	Normal	46	1654	402	
IgG	RA	52	1571	671	<0.0005
	Normal	48	1188	337	
IgA	RA	52	383	242	<0.05
	Normal	47	308	156	
IgM	RA	51	198	106	<0.05
	Normal	47	164	71	
β -1-A	RA	55	98	24	>0.1
	Normal	48	104	37	

Müller and Müller von Voigt (16) have applied a modified Oudin technique for determination of the amount of β 1 A globulin in sera from RA patients. Although the average value was higher than in normal sera the difference was not statistically significant.

The present investigation was aimed not only at a comparison of the levels of IgG, IgA, IgM and β 1 A globulin in the serum of RA patients with the serum levels in normal controls but also at a study of the correlation of these immunoglobulins to each other and to certain other factors such as age, duration of the disease, sedimentation rate, RF titre, antinuclear antibodies and the degree of anaemia.

MATERIAL AND METHODS

Serum samples were obtained from 55 patients suffering from rheumatoid arthritis and from 48 healthy blood donors. All the patients had typical RA by the criteria of the American Rheumatic Association. About half of them were hospital patients, the others attended the outpatients department. The blood donors were selected to represent age groups and a sex ratio similar to those of the RA patients. The patients and the healthy subjects were divided into two age groups, one less than 55 years and one 55 years or more. This age limit was chosen simply because by this means both the patients and the normal controls were divided into two categories of almost equal size.

The serum samples were kept overnight at room temperature and then for 3 days at +4-6°C to complete the *in vitro* conversion of β 1-C to β 1 A globulin (17) and were later stored for varying times at -20°C. The levels of IgG, IgA, IgM and β 1 A globulin were assayed by the single radial immunodiffusion method of Mancini *et al.* (14) with commercially available Paragene plates from Behringwerke Inc., and standard sera from the same

firm. The values given by the producer were not checked, since the main purpose of the study was comparison of the Ig and C levels in RA sera with normal controls, and not the establishment of normal values in a Finnish population. Four of the 12 wells on each plate were filled with 2 μ l of different dilutions of standard sera, and the others with dilutions of the unknown sera. The plates were first kept overnight at 37°C, to preclude errors arising from cryoprecipitation, and then at room temperature. The diameters of the precipitation rings were measured after 2 days with a micrometer magnifier using dark field illumination. In most cases the rings were single, circular and distinct and the standard curves were linear. Results which did not meet these criteria were discarded.

The arithmetical means were calculated, and the significance evaluated by the *t* test. Computer analysis was used for this purpose and for the calculation of correlation factors.

The immunofluorescent tests for detection of antinuclear antibodies were carried out by Dr O. Wager and Dr J. Rasanen of the municipal immunology laboratory at the Aurora Hospital, Helsinki. cryostat sections of mouse liver were used as substrate.

RESULTS

Immunoglobulin and β 1 A globulin levels

It was found that both the total Ig and the IgG levels were significantly higher in the RA series than in normal sera (Table 1 and Fig. 1). Moreover, the IgA and IgM levels seemed to be somewhat higher in the RA series than in the control series, although the difference was not statistically significant in either age group when the two age categories were considered separately (see below under Discussion). No significant difference was noted between the average serum levels of β 1 A globulin.

Age and sex

In the normal series no Ig seemed to be sex dependent, whereas in the RA series the IgA level was significantly higher in males than in females ($p < 0.02$). The total Ig, and the IgG levels (Fig. 1) rose with increasing age in the case of the normal subjects ($p < 0.01$). The IgM level on the other hand was significantly lower in the higher age group. The difference was insignificant when the means of the two main age categories were compared (Fig. 2) but computer analysis revealed a reciprocal correlation between age and serum IgM concentration on the 98 per cent level. No significant correlations between age and Ig values were found in the RA series.

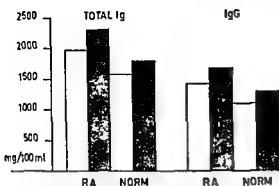


Fig 1 The average total Ig levels and IgG levels in the two age categories. White columns < 55 years black columns ≥ 55 years

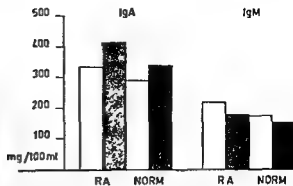


Fig 2 The average IgA and IgM levels in the two age categories. White columns < 55 years black columns ≥ 55 years

Correlations between the immunoglobulins and the β 1 A globulin

Among the normal subjects the β 1 A globulin level seemed to be inversely correlated to the serum level of IgM ($p < 0.05$). In the normal series a positive correlation was found between the serum levels of IgA and total Ig ($p < 0.001$). In the RA series a positive correlation existed between the IgG and IgA levels ($p < 0.001$). An almost significant positive correlation was also noted between both the β 1 A globulin and total Ig levels ($p < 0.05$) and between the β 1 A globulin and IgG levels ($p < 0.05$). No such correlations were found in the normal series.

Other correlations (Table II)

In the RA series the ESR value was positively correlated to the total Ig. The ESR value also seemed to be positively correlated to the IgG and IgA levels and was not very surprisingly reciprocally correlated to the haemoglobin level.

The degree of Latex positivity was correlated to the total level of Ig and also seemed positively correlated to the level of IgG. A correlation could be demonstrated between the degree of positivity of the Waaler Rose reaction and the amount of polyvalent anti nuclear antibody in the patient serum. The amount of IgM type antinuclear antibody in patient serum was positively correlated to the serum level of IgA. A rise seemed to occur in the serum level of IgA with increasing duration of illness while no such correlation was observable in the case of the other Ig's.

DISCUSSION

Various immunological phenomena accompany the disease process in rheumatoid arthritis although their pathogenetic significance is unknown. An effect is to be expected on the circulating levels of the immunoglobulins if the processes are of humoral type and on complement if they require complement factors *in vivo*. However the

Table II Correlation survey RA series

	Total Ig	IgG	IgA	IgM	β -1-A
Duration of disease	—	—	$p < 0.05$	—	—
Hb value	—	—	—	—	—
ESR	$p < 0.01$	$p < 0.05$	$p < 0.05$	—	—
Latex titre	$p < 0.02$	$p < 0.05$	—	—	—
Waaler Rose titre	—	—	$p < 0.05$	—	—
Anti nuclear antibody	—	—	—	—	—
polyvalent	—	—	—	—	—
IgG type	—	—	—	—	—
IgM type	—	—	$p < 0.01$	—	—

No significant correlation

influence on the levels of circulating globulins cannot be predicted, as an increased consumption is probably followed by an increased production and the combined result may be high normal or low levels.

The higher total Ig levels and the IgG levels found in sera from RA patients probably result from immunological stimulation of antibody production. The increase was more pronounced in severe cases with high ESR and low haemoglobin values. The IgA and IgM levels were also increased to some extent in RA patients but only on the 95% level statistically. The correlation between the IgM antinuclear antibody titre and IgA level is surprising, and theoretically difficult of explanation. Even if the correlation was observable on the 99% level it must be stressed that only 13 sera contained such antibodies and the finding may consequently be incidental. The increase in the IgM levels in RA patients was less pronounced than in our previous study in which different techniques were applied. Within the age groups (see below) the difference was not statistically significant. It is well known that in most cases the rheumatoid factor is an IgM globulin and RF titre *in vivo* would probably suppress the circulating level even where the production increased. However no correlation, either positive or negative was found between the Latex or Waaler Rose titre and the IgM level. This observation too is explicable by a proportional effect on the production and on the consumption of IgM.

The influence of age did not prove to be as important as had been reported by Vey and Claessens (24). In the combined series of RA patients and healthy blood donors only the IgG level rose somewhat with increasing age ($p < 0.05$). However we agree with Vey and Claessens that the age factor must be taken into consideration. In the present series the higher IgA and IgM values found in RA patients did not differ significantly from the normals within the age groups. This is somewhat surprising, since the average age of the RA patients was only five years higher than that of the normal controls and the IgA and IgM levels were not found to be significantly correlated to the age in the whole series of RA patients and healthy people. In both age categories the average IgA and IgM levels were higher in RA sera (Fig. 2) but by reason of the smaller numbers of sera tested in the divided material

and the high standard deviations (Table 1) the differences were no longer statistically significant.

The β 1-C globulin, a moiety of C3 was selected for study as representing serum complement factors since serum contains higher amounts of this protein than of other complement fractions, and since it is easily determinable by the radial immunodiffusion technique. The average amount in the whole series (RA and controls) was found to be 100.5 mg per 100 ml. It varied less from case to case than did the immunoglobulins; the standard deviation was 30.9. A slight increase was noted to correlate with increasing total Ig, and with IgG levels ($p < 0.05$) in RA sera. Thus, if there is an increased IgG antibody response the β 1-C globulin production also seems to increase.

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PLASMA VOLUME COLLOID OSMOTIC PRESSURE AND GAMMA GLOBULIN IN MULTIPLE MYELOMA

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Abstract Serum protein concentration, colloid-osmotic pressure and plasma volume have been determined in fourteen patients suffering from multiple myeloma. A positive correlation was found between gamma globulin and plasma volume ($r=0.74$, $p<0.0005$). The colloid osmotic pressure was found to be relatively high, eight out of 70 recordings ranging above 46 cm of water. It is claimed that the relationship between gamma globulin and plasma volume depends on the plasma-expanding effect of gamma globulin. Parallels are drawn to experimental hyperglobulinaemia.

Animal experiments have shown that in hyperimmunized rabbits an increase in gamma globulin is accompanied by an increase in plasma volume (3, 4). In spite of pronounced variations in the gamma globulin concentrations, the colloid-osmotic pressure is normal (6, 9). Most likely this phenomenon is due to the fact that in high concentrations the gamma globulin acts as a "plasma expander" because of its colloid-osmotic pressure. In multiple myeloma, gamma globulin concentrations are found which are similar to those in the above mentioned experiments. The object of the present study was to clarify *inter alia* whether a relationship exists between plasma volume and gamma globulin also in such cases. Furthermore, the colloid-osmotic pressure was studied in order to find out whether it remained normal in spite of the appreciable production of protein in multiple myeloma. Finally, an investigation was made of the relationship between actually measured colloid-osmotic pressure and colloid-osmotic pressure calculated on the basis of the plasma protein concentration in order to see whether an aggregation or a dissociation of the protein molecules takes place. A preliminary report on the study has already been published (6).

MATERIAL AND METHODS

Fourteen patients suffering from multiple myeloma with IgA and IgG myeloma protein were examined in 1967 and 1968. (Most of the patients were examined at Bispebjerg Hospital, Department III. Some of the patients have been examined during their stay at the University Hospital of Copenhagen, Department A, the Copenhagen County Hospital in Gentofte, Department C, and the Finsen Institute, Copenhagen Medical Department.) The sex and age of the patients are shown in Table I. All the patients were examined for the purpose of revealing conditions if any which might influence the plasma volume. In particular, examinations aimed at revealing cardiac failure and gastrointestinal disorders were carried out.

The blood samples were drawn in the morning with the patient lying down. Serum gamma globulin was determined by paper electrophoresis, adopting the method of Laurell et al. (10). No correction was used for the varying stainability of gamma globulin. Gamma globulin was taken to represent myeloma protein plus normal gamma globulin. The total protein determination was made using a biuret method. Plasma volume was determined as the 10 min distribution volume of intravenously injected ^{125}I labelled serum albumin or Evans Blue.

Colloid-osmotic pressure (c.o.p.) in serum was determined by the Tybjaerg-Hansen method (8). (The determinations were carried out at the Department of Clinical Physiology, Bispebjerg Hospital.) Colloid-osmotic pressure was calculated from the equation

$$\text{c.o.p.} = 1.33 \times (4.20A + 0.33G + 2.35G)$$

where A is the serum albumin and G the serum gamma globulin concentration (6). The coefficient of variation is 10.

RESULTS

The results appear in Table I, presenting one measurement from each of the 14 patients. It will be seen that the plasma volume increases with increasing levels of gamma globulin. Table II shows all measurements from the five patients in

Table I Serum gamma globulin colloid osmotic pressure and plasma

No	Sex	Age	Type of myeloma protein	Weight (kg)	Hæmoglobin (g/100 ml)	Serum albumin (g/100 ml)	Serum gamma globulin incl paraprotein (g/100 ml)	Colloid osmotic pressure (calculated cm of water)	Colloid osmotic pressure (observed cm of water)	Plasma volume (ml)	Plasma volume (ml/kg)
1*	♀	58	IgA	71.5	7.4	3.28	1.72	78.5	49.1	1390	47.4
2	♀	67	IgG	72.5	9.0	1.17	2.78	32.6	37.4	1270	45.2
3	♀	70	IgA	63.0	10.0	4.45	4.5	47.5	42.1	2990	47.4
4	♀	63	IgG	95.0	8.2	3.71	3.06	36.4	37.0	3610	38.0
5	♀	56	IgG	71.6	11.9	3.96	1.61	40.3	45.0	2490	34.8
6	♀	54	IgG	74.0	9.4	4.15	1.95	43.1	44.7	2530	34.2
7	♂	62	IgG	62	16.0	3.51	68.0	38.1	43.4	1700	47.0
8	♀	70	IgG	55.2	9.5	2.89	5.46	37.0	42.9	1860	51.8
9	♂	37	IgG	39.2	10.7	3.92	6.12	46.9	44.4	1800	62.3
10	♂	73	IgA	52.0	9.8	2.77	6.40	38.9	45.9	1610	69.8
11	♀	60	IgG	60.6	10.8	3.07	7.0	44.0	41.8	2870	47.4
12	♀	71	IgA	73.0	5.9	1.78	9.13	40.4	44.3	4030	55.4
13	♂	64	IgA	69.0	6.3	3.12	9.15	50.5	51.8	5530	80.2
14	♂	81	IgG	53.0	5.8	2.95	11.06	55.0	53.8	36.0	68.3

Uracemia

Table II Serum gamma globulin colloid osmotic pressure and plasma volume studied two to four times in five cases of multiple myeloma

No	Date	Weight (kg)	Haemoglobin (g/100 ml)	Serum albumin (g/100 ml)	Serum gamma globulin incl paraprotein (g/100 ml)	Colloid osmotic pressure (calculated cm of water)	Colloid osmotic pressure (observed cm of water)	Plasma volume (ml)	Plasma volume (ml/kg)
4	30.8.67	104.5	5.9	3.24	2.13	29.4	28.1	5310	58.1
—	13.9.67 ^a	95.0	8.2	3.71	3.06	36.4	37.0	3810	38.0
6	11.9.67	74.0	9.4	4.15	3.95	43.1	44.7	2330	34.2
—	21.9.67	74.0	9.8	4.15	3.95	43.1	46.7	2340	31.6
7	9.5.68	68.0	10.8	3.31	4.16	38.1	43.4	3.00	47.0
—	6.6.68	68.0	6.8	2.36	6.50	36.0	44.8	4210	63.7
9	6.4.68	61.0	10.7	3.92	6.12	46.9	44.4	3800	62.3
—	17.6.68	65.0	13.5	4.41	4.59	47.7	45.3	3330	51.2
12	8.4.67	73.0	5.9	1.78	9.13	40.4	44.3	4030	55.4
—	10.5.67	70.0	6.9	2.32	6.10	34.4	34.4	31.0	44.5
—	8.6.67	68.9	7.1	—46	2.77	25.1	25.8	590	39.0
—	6.7.67	67.5	10.6	3.61	1.85	31.7	26.5	670	39.6

Incompensated heart disease

At birth, the mean plasma volume (500 ml) given on July 6, 7 and 8

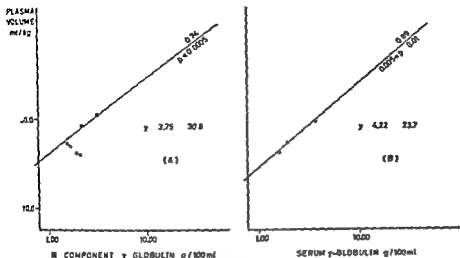


Fig 1 (A) The relation between plasma volume and gamma globulin (normal gamma globulin + paraprotein) in multiple myeloma (0 analyses in 14 patients) (B) The

relation between plasma volume and gamma globulin in hyperimmunized rabbits

whom two or more measurements were taken during the course of the disease. In patients 6, 7, 9 and 12 the plasma volume tends to vary with changes in the concentration of gamma globulin. This is particularly evident in patient 12 on whom several tests were made during the course of the disease. Patient 4 is an exception. In this case the highest plasma volume was found at a time when the patient suffered from severe cardiac failure with oedema whereas the lowest plasma volume was measured when, after appropriate treatment, the failure had been alleviated. The corresponding gamma globulin values were 2.13 and 3.06 g per 100 ml respectively and hence they varied inversely with the plasma volume. All interdependent plasma volumes and gamma globulin values are shown graphically in Fig 1(A) with the exception of those measured in patient 4 for whom only one value recorded while the heart disease was well compensated is given. It will be seen that a distinct correlation exists between the plasma volume and the concentration of gamma globulin. The correlation coefficient is 0.74 ($p < 0.0005$). The equation for the regression line is $y = 3.75x - 30.8$.

The correlations between the haemoglobin concentration and the plasma volume and between the albumin concentration and the plasma volume were also calculated. In both cases slightly negative correlations were found (haemoglobin and

plasma volume $r = -0.38$ $p < 0.05$; albumin and plasma volume $r = -0.38$ $p < 0.05$).

In two patients (no 7 (6668) and no 13 (19768)) the central venous pressure was measured and found to be normal, 10 and 9 mm Hg, respectively at a plasma volume of 63.7 and 80.2 ml/kg.

The measured colloid-osmotic pressure was found to range between 25.8 and 53.8 cm of water. The calculated colloid-osmotic pressure varied from 25.1 to 55.0 cm of water.

DISCUSSION

The study revealed a positive correlation between gamma globulin concentration and plasma volume in patients suffering from multiple myeloma. This positive correlation can be observed also in the individual patients in connection with changes in the gamma globulin concentration. The patient (no 4) who suffered from cardiac failure is an exception as the plasma volume under these conditions increases with cardiac failure and decreases when the failure is alleviated. Presumably this phenomenon explains why it has not been possible to demonstrate earlier any correlation in materials published by Gabuzda (7) and Andersen (1). However, when the case reports included in Andersen's material are analysed and one patient with cardiac disease is excluded, there seems to be a

correlation (6). The literature relating to this subject is however scarce. Birke (2) mentions briefly that there is a highly significant increase of plasma volume in hypergammaglobulin cases but apart from this finding no other mention appears to have been made. As stated above animal experiments have shown that in hyperimmunized rabbits there is a positive correlation between gamma globulin and plasma volume. Fig. 1(B) presents results reported previously (4) obtained in 13 hyperimmunized rabbits. The correlation coefficient is 0.88 ($p < 0.01$) and the equation for the regression line is $y = 4.22x + 23.7$. The difference in slope between the regression line for patients with myeloma and immunized rabbits is not significant. Hence these experimental results seem to be parallel to the conditions found in multiple myeloma.

There is a possibility that the production of gamma globulin in multiple myeloma might reach levels of such a height that an overloading would occur with a high central venous pressure similar to the conditions seen in connection with excessive transfusions of blood and plasma. How normal central venous pressure was observed in two patients with a very high plasma time.

In the present material the colloid-osmotic pressure was found to be surprisingly high, eight out of 20 readings being definitely above 46 cm of water, which is the upper normal limit for the method employed. A comparison of measured and calculated colloid-osmotic pressure shows that in one case (patient 12, 26.7.67) the measured pressure was slightly below the calculated pressure and in another (patient 1) the measured pressure was considerably higher than the calculated pressure.

It is thought that the difference between measured and calculated colloid-osmotic pressure may be explained by aggregation of protein molecules. The patient in whom a lower measured than calculated pressure was found did not present any clinical signs of aggregation of protein molecules associated with the hyperviscosity syndrome (11). The size of the molecules was not determined by ultracentrifugation. The other patient in whom the measured pressure was considerably higher than the calculated, suffered from severe uraemia presenting a serum creatinine level of 12 mg per 100 ml. Our continued studies will show whether

uraemia exerts any influence on the determination of the colloid-osmotic pressure with the method employed.

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THE CONTROL OF PLASMINOGEN AND PLASMIN DURING THROMBOLYTIC THERAPY

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Abstract Plasminogen levels during thrombolytic therapy have been recorded utilizing a previously standardized method. The plasminogen is determined as proactivator in a rapid clot lysis system. The method seems to be reliable and may be used as an alternative to the caseinolytic assays.

Plasminogen and plasmin are important factors in the modern principles of streptokinase treatment. A marked fall in plasminogen and subsequent rapid elimination of plasmin from circulation is desired, in order to avoid proteolysis of plasma components (7, 11, 22). The mechanism in urokinase treatment may possibly be somewhat different, but this is not finally established (8).

In the present work a previously described method for determination of human plasminogen, probably also including plasmin, has been used (2, 3). The assay is based upon a clot lysis system and has proved valuable as a rapid evaluation of the plasminogen (and plasmin) level during thrombolytic therapy with streptokinase and urokinase.

MATERIAL

Anticoagulant Sodium citrate dihydrate 3.13%. For clotting studies the citrate solution contained 5 mg of epsilon-amino-caproic acid and 2500 units of Trasylol per ml in order to avoid fibrinogenolysis *in vitro*. These concentrations of inhibitors did not influence the clotting systems.

Buff = A modified veronal buffer (pH 7.35 and ionic strength 0.15) (1).

Streptokinase (Streptase) from Hoechst, Behringwerke AG Marburg/Lahn, Germany (topostasin) from Roche, Basel, Switzerland (expressed in NIH units) and urokinase from Roche, Basel, Switzerland (expressed in C.T.A. units).

METHODS

Collection of blood Nine parts of blood were mixed with one part of anticoagulant at 4°C. Plasma was prepared by centrifuging at 4°C for 30 min at 1400 rpm (1400 g) and stored in aliquots at -70°C.

Fibrinogen was determined as fibrin after coagulation with thrombin by the method of Jacobsson (13) with the modification of Blomback and Blomback (5) and Godal (9). For the collection of blood anticoagulant with addition of fibrinolytic inhibitors was used (see Material).

Plasminogen determination

1. Plasminogen was determined as proactivator (P.A.) by the standardized method of Blix (3). 0.10 ml citrated bovine plasma #05 ml streptokinase (8000 U/ml buffer), 0.05 ml citrated human test plasma (diluted 1:10 in buffer), 0.10 ml thrombin (30 U/ml buffer). If desirable all volumes may be doubled.

All reagents were kept on ice water. The bovine plasma was incubated for 15 sec. at 37°C in glass test tubes (10 x 70 mm) and the other materials were added at 15 sec intervals. The time from addition of thrombin to complete lysis of the clot was recorded. Soon after the clot had formed, small air bubbles could be observed in the clot, as described by Blomback, et al (4) and Lassen (17). A few seconds before the clot had been lysed, all the air bubbles rose to the surface. When they reached the surface the clot was completely lysed and this point was easy to record. The test tubes should not be tilted but carefully raised at intervals for inspection of the air bubbles. All tests were performed in duplicate.

The per cent of plasminogen (proactivator) is read on a reference curve obtained from dilution series of pooled normal plasma (Fig. 1). Due to the slope of the curve plasmas with normal or higher concentrations of plasminogen might better be tested in dilution 1:20 rather than 1:10 and the per cent value multiplied by 10.

The most convenient lysis time at the 100% level is between 120 and 180 sec. Some bovine plasmas will be too long lysis times but this can be corrected by increasing the concentration of thrombin (*).

2. Plasminogen was also determined by a caseinolytic method after acidification of plasma with HCl and sub-

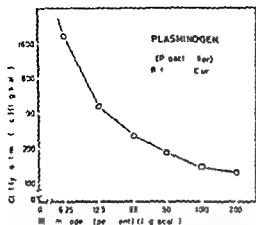


Fig 2 The reference curve for calculation of the plasminogen (proactivator) concentration in human plasma. The abscissa gives the values in per cent of normal obtained by dilution series of pooled, normal human plasma in buffer (100% is plasma tested in dilution 1:10).

sequent neutralization with NaOH. A 1% casein solution was used as substrate and the plasminogen converted to plasmin by means of urokinase. The incubation period was 60 min.

Thrombin time was recorded in this system. 0.2 ml citrated plasma, 0.1 ml thrombin (6 U/ml saline). Normal clotting time 13–15 sec.

For the collection of blood anticoagulant with addition fibrinolytic inhibitors was used (see Material).

ADMINISTRATION OF THE THROMBOLYTIC THERAPY

Administration of streptokinase

Three patients are presented in this report. The initial dose was 250 000 units in 20 ml saline during 15 min.

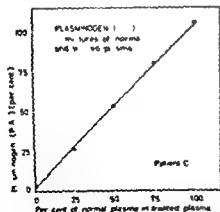


Fig 3 Mixtures of plasmas from a patient before and during treatment, as tested in the present plasminogen system (ordinate). The plasminogen in the treated plasma was found to be 3.5% when controlled by a caseinolytic method (see text).

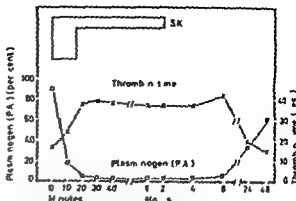


Fig 3 Plasminogen and thrombin time in a patient (A) treated with streptokinase for two hours.

The maintenance dose in two patients was 150 000 units per hour by continuous infusion. In the third patient this dose was reduced to 100 000 units per hour after six hours. Just prior to the initial dose 100 mg of hydrocortisone (Actocortin) was given intravenously and the treatment was followed by oral anticoagulant therapy. No bleeding complication was seen and the Ivy bleeding time remained normal during the whole period of treatment in all patients.

2 Administration of urokinase

The urokinase investigation was carried out by H. Arnesen, M.D., in this laboratory. The initial dose was about 250 000 units (3600 U/kg) in ten minutes and the maintenance dose the same amount per hour by continuous infusion. This treatment was also followed by anticoagulant therapy and no complications were seen.

INVESTIGATION AND RESULTS

1 Plasminogen determination in plasma containing various amounts of plasminogen

The plasminogen level in plasma from a patient (C) one hour after beginning of the maintenance dose determined by a caseinolytic method was found to be 3.5%. This plasma was mixed in various proportions with plasma collected from the same patient before treatment and the plasminogen levels were determined by the present clot lysis method. The results are given in Fig. 2. There is a good agreement between the calculated (abscissa) and the recorded (ordinate) values.

2 Plasminogen determination during streptokinase treatment

Three patients (A, B, C) are presented and the results of plasminogen determinations and thrombin times are given in Figs. 3, 4 and 5. Very low

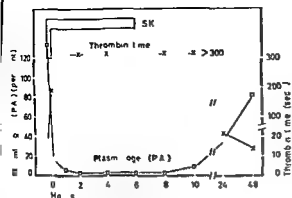


Fig 4 Plasminogen and thrombin time in a patient (B) treated with streptokinase for six hours

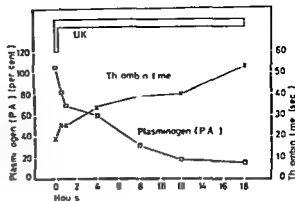


Fig 6 Plasminogen and thrombin time in a patient (D) treated with urokinase for eighteen hours

plasminogen values were recorded in all patients after the initial dose had been infused. After 48 hours the plasminogen values had still not reached the pretreatment levels. In patient B there was no detectable fibrinogen in the patient's plasma when the thrombin time exceeded 300 sec.

3 Plasminogen determination during urokinase treatment

One patient (D) is reported and Fig 6 illustrates a different pattern of plasminogen decline than during streptokinase treatment.

DISCUSSION

The caseinolytic method (16-21) has frequently been utilized for determination of plasminogen but may be less convenient in routine laboratories due to the necessity of highly standardized systems

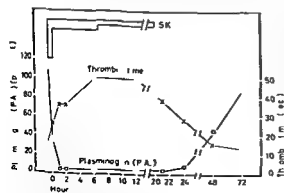


Fig 5 Plasminogen and thrombin time in a patient (C) treated with streptokinase for twenty hours

(10-12). The application of fibrin plates (1) for this purpose (19) is not practical during thrombolytic therapy.

Christensen (6) suggested a clot lysis method introducing bovine fibrinogen as substrate and human serum in combination with streptokinase as test material. The mechanism was better understood when Mullertz and Lassen (18) discovered a factor proactivator which reacted stoichiometrically with streptokinase in the formation of an activator of plasminogen. The isolation of proactivator from human plasminogen has never been successful, however, and there is reason to believe that it is a property connected with the plasminogen molecule itself. Plasmas of most animal species do not contain this proactivator property of plasminogen which makes them suitable as enzyme substrate in proactivator assays.

Johnson and Tillet (14), Blomback et al (4) and Lassen (17) utilized this principle in clot lysis systems for the quantitative determination of plasminogen as proactivator. The present method was based upon the same principle using bovine plasma instead of fibrinogen as substrate (2). It was found to be suitable for determination of plasminogen in fibrinolytic human plasmas, probably giving the combined content of plasminogen and plasmin (3). Kontinen et al (15) found bovine plasma useful for such assays, although in some respects it differed from purified fibrinogen. On the other hand, de Vreker (23) preferred fibrinogen in his modified system.

The results obtained with the present method seem to be reliable, giving a rapid answer with a high reproducibility when used in patients during

thrombotic therapy. In all the streptokinase treated patients plasminogen decreased to less than 6% of normal during the initial period and remained at this level till the end of therapy. More than 48 hours was necessary to restore pretreatment values. Plasminogen decline in the urokinase treated patient was much slower and the level never fell below 15%.

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A NEW TECHNIQUE FOR DYNAMIC SPIROMETRY ON PATIENTS WITH CONTAGIOUS RESPIRATORY DISEASES

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Abstract A system with a disposable plastic bag in a box between the patient and the Bernstein spirometer is described which eliminates the risk of spreading infections between patients via the spirometer. The present modification increased the amplitude response of the spirometer by about 3 l at 100 c/min the random error was not increased.

The analysis of the ventilatory function in patients with acute or chronic contagious diseases of the lungs or airways e.g. tuberculosis mycoplasma mycoides or purulent bronchitis involves some problems concerning disinfection of the spirometer. Even if the spirometer is cleaned after each patient, which generally is not done it is not possible to guarantee a safe spirometer on all occasions without excessive labour for each cleaning procedure. It is worse however if the infectious character of the disease is not suspected at the time of investigation and the spirometer therefore is not specially cleaned. It is also unsatisfactory to refuse a patient from study until for instance a negative guinea pig inoculation test for tuberculosis has been obtained which may take up to six weeks. There seems to be a need for a modification so that dynamic spirometry can be performed routinely on contagious patients without undue risks for other patients and undue labour for the laboratory personnel.

In the present study a modification of dynamic spirometry is described in which the patients breathe in and out of a disposable thin walled plastic bag enclosed in a plexiglass cylinder which communicates with the spirometer. The system has been studied in model experiments and tested on patients and is easy to use in routine practice.

METHODS AND MATERIAL

The modification is described in Fig 1. Through a rubber mask and a large bore rubber tube (A) the patient breathes in and out of a thin walled (0.02 mm) plastic bag (B 40 x 50 cm effective volume about 13 l) attached by an O-ring to a groove in the metal cylindrical part C. This siliconized O-ring seals the plastic bag airtight in a plexiglass cylinder (D) which communicates with a slightly modified Bernstein spirometer (E) (Kifa AB Solna) (1). The cone (F) prevents the air stream from the spirometer from turning the plastic bag inside out and causing an obstruction at C. The tap (G) on top of the plexiglass cylinder helps to adjust the proper volume level of the spirometer.

The plastic bag, the rubber mask, the tube and the metallic part C are changed after each test, all parts but the plastic bag being heat sterilized.

The dynamic characteristics of the system were objectively tested by driving it with a 15 l pneumatic piston (E. Bohlin, Stockholm) at various frequencies. The system was also tested on one group of normal subjects and one group of patients with obstructive pulmonary diseases. The normal group comprised six men and nine women with an average age of 29 years (range 15-67). The patient group consisted of ten men and one woman with an average age of 56 years (range 32-68) all with clinical signs of airway obstruction. Both groups were studied twice on separate days, half of each group starting with the normal spirometer, the other half starting with the plastic bag and plexiglass cylinder inserted between the patient and the spirometer. For each study three forced inspiratory and expiratory vital capacities were recorded and two maximal voluntary ventilations at each frequency. For each measurement the highest value was used.

Statistical calculations were performed according to Snedecor (7). The following probability (P) levels of significance were used: $P < 0.01$ significant and $P < 0.05$ probably significant.

RESULTS

The amplitude responses at varying frequencies of the spirometer with and without the present mod-

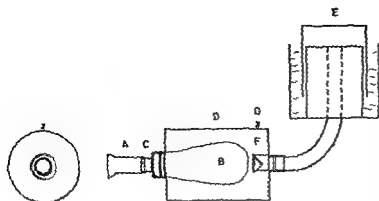


Fig 1 A modification of dynamic spirometry see text. A rubber mask and tube B plastic bag C metal cylindrical part with O ring for sealing the plastic bag in the plexiglass cylinder D plexiglass cylinder E Bernstein spirometer F cone G tap for volume control

Table 1 Some ventilatory capacity tests and the difference between the values obtained with the present modification and with the spirometer alone (diff)

P = probability that the differences are caused by random factors C = coefficient of variation the error of a single determination in per cent of the mean value

	Control group <i>n</i> = 15				Patient group <i>n</i> = 11			
	Mean	Diff	P	C	Mean	Diff	P	C
VC	4.29	0.047	>0.0	2.5	3.18	0.013	>0.70	3.0
FEV 10	3.72	0.090	<0.05	2.8	1.34	0.017	>0.30	2.5
FEV	87	1.3	>0.05	2.2	47	0.5	>0.40	3.4
FIV 10	4.04	0.043	>0.30	3.3	2.69	0.043	>0.40	4.3
FIV	94	0.1	>0.70	1.1	84	1.3	>0.50	3.6
VV 40	122	0.7	>0.30	1.7	54	0.1	>0.95	9.5
VV 100	160	4.3	<0.05	3.2	62	1.3	>0.50	7.8
VV 150	161	2.8	<0.01	5.5	63	1.3	>0.40	6.1

sification were tested with the pneumatic piston and are shown in Fig 1. With the spirometer alone the amplitude response increased successively with rising frequency, the rise from 26 c/min to 126 c/min being 13%. With the present modification the corresponding amplitude increase was 19% at the frequency of 146 c/min. At the frequency of 96 c/min the amplitude increase

with the spirometer alone was 6% and with the present modification 9%. The distortion of the curves at higher frequencies is evident from Fig 2, slightly more marked changes were noted with the plastic bag attached.

The effect of the present modification on the

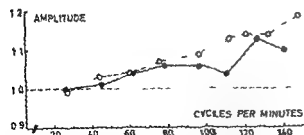


Fig 2 Amplitude responses of the regular (—●—) and the modified spirometer (---○---) at varying frequencies when tested with a motor-driven 15 l pneumatic piston. Each symbol represents the average of 10–20 consecutive cycles.

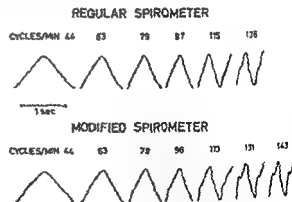


Fig 3 Volume curves at varying frequencies when tested with a motor-driven 15 l pneumatic piston.

different variables of dynamic spirometry in the control group and the patient group is given in Table I. The maximal voluntary ventilations were the only variables that were significantly changed being slightly larger when recorded with the plastic bag system. These small absolute changes seem to be related to the increased amplitude response at higher frequencies as shown in Fig. 1. As expected the coefficient of variation for the different variables was less in the control group than in the patient group.

DISCUSSION

With the present technique of heat sterilization or exchange of all parts in contact with the expired air a safe spirometer can be guaranteed for each patient although used for subjects with tuberculosis, mycoplasma, mycosis or other infectious diseases. This should be of special value in departments for infectious diseases and for tuberculous patients but also in hospitals with a mixed clinical material. In routine practice the procedure with this modification takes only 2-3 min extra which is much less than any procedure for cleaning the spirometer.

The present study shows that this modification gives a slight systematic error with a somewhat more pronounced distortion of the amplitude response at higher frequencies. However at frequencies below 100 c/min and for practical purpose even up to 140 c/min this difference is quite negligible.

The random error of the method as computed from the difference between the spirometric values obtained with and without this modification was in the control group ($1-3^\circ$) similar to the values given for dynamic spirometry by Berglund et al (2) but lower than those given by Ringqvist (5). In the patient group the error ($3-9^\circ$) was larger than the values given for patients with sarcoidosis (3) but less than those given for patients with bronchial asthma (4) and chronic bronchitis (6). Therefore no random error seems to be introduced with the present modification.

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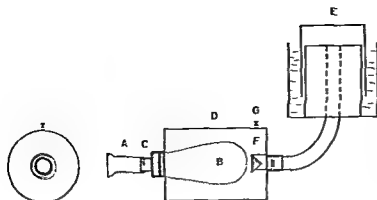


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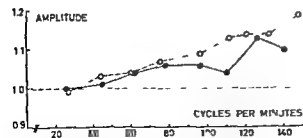


Fig 2 Amplitude responses of the regular (●—●) and the modified spirometer (○—○) at varying frequencies when tested with a motor-driven 1.5 l pneumatic piston. Each symbol represents the average of 10–20 consecutive cycles.

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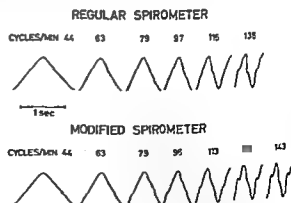


Fig 3 Volume curves at varying frequencies when tested with a motor-driven 1.5 l pneumatic piston.

IRON ABSORPTION IN APPARENTLY HEALTHY MEN AND WOMEN

III Studies in Iron Absorption¹

S Hoglund

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Abstract Thirty three women and 24 men all apparently healthy have been investigated in respect of iron absorption by means of radioactive iron and a whole body counter. Six out of 33 women had either a somewhat low serum iron concentration or a high TIBC and in nearly 40% of them absorption exceeded the upper limit for the men. The mean absorption of the women was about double that of the men. No correlation could be shown to exist between absorption on the one hand and hemoglobin, packed cell volume, serum iron and TIBC on the other.

The introduction of the whole body counter into clinical medicine has simplified the study of iron absorption and has made it possible to carry out routine investigations of iron absorption as a link in the diagnosis of sideropenia, intestinal disturbances, diseases of the blood etc. Earlier methods of measuring iron absorption have been reported elsewhere (8) and have proved to be very laborious for clinical use.

The aim of this investigation was to present values of iron absorption for both men and women of an apparently healthy condition. A summary of so-called normal values previously reported is given in Table I.

MATERIAL AND METHODS

Thirty three healthy women between the ages of 20 and 41 (mean age 26.5) and 24 healthy men between the ages of 21 and 50 (mean age 28.6) were studied. In the histories of the persons investigated there was no record of abnormal bleeding, anemia, menorrhagia or iron therapy during the year prior to investigation. None of them had been a blood donor. None had consulted a physician or received medical treatment and all of them

were working. None of the women had been pregnant during the last year.

The subjects were instructed to fast for 10 hours before and 2 hours after administration of the test dose. In nine of the women hemoglobin concentration and packed cell volume were measured. Twelve of the female subjects were investigated for serum iron concentration and total iron binding capacity (TIBC) and 21 only for serum iron. In nine of the male subjects hemoglobin concentration and packed cell volume were investigated in five serum iron and TIBC and in seven only serum iron.

Table II shows the normal values and the error in the determination of serum iron and TIBC performed at our own laboratory. Serum iron was determined according to Agner's method in which the protein-bound serum iron, after acidifying to pH 2.5-3.0 reacts directly with *o*-phenanthroline without previous hydrolysis and precipitation of protein. The color intensity was subsequently determined colorimetrically (1). For TIBC determination, transferrin was first saturated with ferric chloride and surplus iron ions were precipitated by magnesium carbonate after which the transferrin-bound iron was determined as serum iron (13). From the serum iron values transferrin values can readily be deduced. At the beginning of the investigation some serum iron and TIBC determinations were made at the Central Laboratory for Clinical Chemistry Karolinska Hospital.

In order to reduce the effect of individual variations in the absorption of iron during the day the absorption tests were made between 9 and 9.40 a.m.

Iron absorption was investigated by means of radioactive iron. 0.5 mg of iron was given by mouth in the form of ferrous sulphate labelled with 0.5-1 μ Ci ^{59}Fe in a small quantity of distilled water. The beaker used for 1 mg the test dose was rinsed 3 times with distilled water which the subjects were asked to swallow so as to make certain that the full test dose was administered.

The radioactivity of the body was measured with the aid of a whole body counter (11). This consists of a measuring chamber 20 x 20 x 205 cm, built on a mounting of low activity material which is known as *hofsiste* with walls of steel plate 15 cm thick. The measuring instrument consists of sodium iodide crystals 5 x 3 inches

¹ Presented in part to the Panel of the International Atomic Energy Agency Vienna 1965.

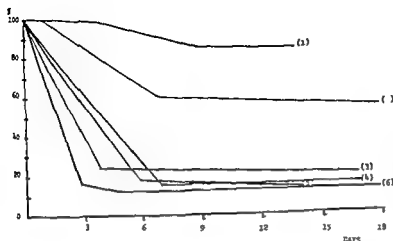


Fig 1 Whole body radioactivity after oral administration of 0.25 mg ferrous sulphate labelled with 0.5-1 μ Ci ^{59}Fe to two women (curves 1 2) and four normal men (curves 3-6) One of the women (1) had somewhat low serum iron

vestigation was made this value was taken as the basis for the changes in the body's radioactivity due to the radioactive material supplied

The whole body activity was measured prior to administration of radioactive iron and also 1 hour after

The dose retained after 13-20 days was regarded as absorbed, and it was assumed that excretion of already absorbed iron could be disregarded Measurement times and physical data are given in Table III

RESULTS

Repeated measurements of whole body activity on the days following the administration of the test dose showed that the values were greatly diminished during the first 3-4 days corresponding to the activity eliminated from the body in the feces Fig 1 shows how approximately after 7 days the values become stabilized After 10 days hardly any of the test dose was excreted

Out of the 33 selected healthy women six had serum iron values below 0.070 mg% or TIBC values above 0.400 mg%

In Table IV the results of the iron absorption investigation are given Substantial differences in iron absorption between women and men were obtained The higher absorption in women was found to be statistically significant ($0.01 > p > 0.001$) This applied also to the women whose serum iron values were above 0.070 mg% and TIBC values below 0.400 mg%

Mean iron absorption in the women was 2.3 times higher than that in men which means that the women on an average absorbed 25% more of the given dose

Thirty six per cent of the women had a higher iron absorption than the man with the highest value which was 41% There was no statistically significant correlation between individual values of hemoglobin packed cell volume serum iron TIBC and iron absorption (Table V) Dispersion of the absorption values was so wide that even very low values were within the normal limits

DISCUSSION

Method

The low test dose of 0.25 mg was chosen because it was assumed to give rise to a physiologic amount of free iron ions in the small intestine

Values subsequently obtained were compared to the total radioactivity of the body after the administration of the test dose (In order to avoid

Table IV Normal iron absorption (per cent of given dose)

Subject category	No of subjects	Mean	S.E.
Male	24	19.0	2.3
Female entire group	33	43.5	4.4
Female serum iron > 0.070 mg% TIBC < 0.400 mg%	27	39.7	4.5
Female serum iron < 0.070 mg% TIBC > 0.400 mg%	6	60.5	17.2

Table V Correlation coefficients and regression coefficients between iron absorption and hematological variables in healthy fertile females

Clinical variable	No of subjects	Correlation coefficient	Regression coefficient	Statistical significance of correlation
Hemoglobin concentration g/100 ml	9	-0.489	-3.24×10^{-4}	$p > 0.05$
Packed cell volume	9	-0.314	-3.98×10^{-4}	$p > 0.05$
Serum iron mg/100 ml	27	0.152	2.05×10^{-4}	$p > 0.05$
TIBC mg/100 ml	9	0.541	3.8×10^{-4}	$p > 0.05$

losses in the feces this initial value was measured after one hour.)

The distribution of the isotope in the body during the time immediately after administration may cause the degree of efficiency to vary when measurements are made. It has been shown that for a whole body counter with a one crystal and chair geometry 4 hours after administration the degree of efficiency is comparable to that obtained after 2 weeks (12-17). The dependence on geometry of the apparatus in which scanning geometry is utilized is however only between $1/4$ to $1/10$ of that of the apparatus in chair geometry as applied (11). Therefore the initial values were measured one hour after administration of the test dose in the present investigations. Even small losses of radioactive iron in the feces can then be avoided.

If unabsorbed iron is excreted for more than 10 days after administration the method used may give too low absorption values. After about 10 days however it seems that only 0.5% of the administered dose is eliminated via the intestine (2). This also indicates that the amount of radioactive iron already absorbed which can leave the body in this way during the time the measurements are made must be very small although a certain excretory function with regard to iron has been ascribed to the cells of the intestinal mucosa (5).

On account of the short life cycle of the cells of the intestinal mucosa (2-4 days) (4) it is possible that iron taken up by these cells returns to the lumen of the intestine inasmuch as the cells are desquamated. Whether this iron was absorbed is a semantic question (15). After 10-14 days when retention was measured the cells of the intestinal mucous membrane have probably been renewed once or twice and it is likely that

the amount of iron due to the test dose which can be recovered in the mucosal cells is small.

Results

Serum iron and TIBC values seem to be very widely dispersed normally (Table II) and the somewhat low serum iron and high TIBC values found in some of the women are not surprising. They are also in good agreement with earlier reports in the literature (14-20). Single determinations of serum iron and TIBC thus seem to be of limited value as criteria of iron metabolism. Furthermore our investigations show that in most women there is an increased absorption from the gut of iron concentrations likely to occur physiologically. This is in agreement with the recent reports by Heinrich et al. (7). Since no correlation was found between any of the parameters hemoglobin, packed cell volume, serum iron, TIBC and iron absorption, intestinal absorption of iron seems to act independently of these factors. The relation between such humoral factors and iron absorption have been further investigated and the results separately published (9-10).

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ON THE MECHANISM OF RENIN STIMULATION

The Effect of Postural Change Salt Depletion and Exercise

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Abstract The correlation between hemoconcentration (expressed by increase in colloid osmotic pressure (Δ COP)) and plasma renin activity increase (Δ PRA) has been investigated during 1) change of posture from supine to standing position in normals, who were salt-depleted the three previous days (10 mEq Na per day on the third day 100 mg chlorthalidone (Hygroton[®])) and 2) in exercise experiments on normals on liberal salt intake in sitting position on bicycle ergometer (medium work load-terminal pulse rate 140-140). The correlation Δ COP- Δ PRA was in 1) linear and significant, $r=0.83$ $p<0.001$. The slope of the line was significantly steeper than in corresponding experiments on liberal salt intake (PRA increase per unit COP increase was greater). Mean supine PRA was significantly higher than on liberal salt intake but the range overlapped. In 2) a significant PRA increase in all experiments appeared but no correlation Δ COP- Δ PRA. It is concluded that salt depletion of some days duration (chronic) sensibilizes the renin releasing mechanism for the postural stimulus. The lack of correlation Δ COP- Δ PRA for the exercise studies suggests a mechanism for renin stimulation different from the one in postural shift and acute salt depletion.

In a previous paper (11) the relationship between hemoconcentration (expressed by increase in colloid osmotic pressure (COP)) and increase in plasma renin activity (PRA) from supine to erect position in normal humans on liberal salt intake has been studied. A significant linear correlation between Δ COP and Δ PRA was found. Acute salt depletion (furosemide) of supine normals demonstrated a similar significant correlation. The two regression lines were not significantly different. Drawing of blood in volumes (435 ml) corresponding to plasma volume decrease in the furosemide experiments from supine normals gave no increase in PRA. It was concluded that PRA increase on change of posture and in acute salt depletion was a function

of hemoconcentration. It was therefore found of interest to investigate the same relationship during changes of posture in salt-depleted normal individuals and during exercise in normals on liberal salt intake in order to study whether the renin release which is known to take place (3, 4 and 8) could be explained in the same manner under these conditions.

MATERIAL AND METHODS

Studies on changes of posture during salt depletion were carried out in ten individuals aged 22-61 years, six males and four females. All were hospitalized for minor conditions unrelated to the cardio-vascular or renal system. No drugs were given prior to the investigation.

Studies during exercise on a bicycle ergometer were carried out in seven trained individuals aged 22-40 years, six males and one female. Blood samples were obtained by placing an indwelling needle in an antecubital vein; coagulation in the needle being prevented by flushing it with small amounts of 3.8% Na citrate. Immediately before the blood samples were obtained a few ml of blood were drawn and discarded because of contamination with Na citrate from the needle. Subsequently blood samples were drawn into two disposable syringes of 10 ml each containing 1 ml of Na citrate. The blood was transferred to a siliconized Erlenmeyer flask immersed into ice water. Within one hour the blood was centrifuged at 0°C 3000 rpm for 15 min. The plasma was separated and kept at -20°C until renin activity was determined. The renin activity was determined by the method of Boucher et al slightly modified (coefficient of variation $\pm 11.8\%$) (10). The results are expressed as ng angiotensin/10 ml of plasma/4 h of incubation. In addition to the blood obtained for measurements of renin activity another 3 ml of blood was drawn into a dry syringe for determination of colloid osmotic pressure. COP was recorded in an electronic osmometer for quick direct measurement of small samples described by Hansen (7). The results are expressed in cm H₂O. Confidence limits 95% ± 0.5 mm Hg. Plasma volume in the stand-

Table I Postural experiments after salt depletion

0=sample after 45 min of recumbency 5, 20 and 30 min after assumption of the standing position
 Mean standing values are obtained as average of 20 and 30 min values

Case no	Sex	Age (y)	PRA ₀	PRA ₅	PRA ₂₀	PRA ₃₀	Mean standing PRA	Standing plasma volume in of supine
			COP ₀	COP ₅	COP ₂₀	COP ₃₀	COP	
1	♂	41	35 42.7	60 45.9	90 48.3	104 51.4	97 49.9	85.6
2	♂	31	84 51.2	125 59.4	177 59.0	169 57.7	173 58.4	87.7
3	♂	23	148 50.5	208 54.7	216 56.5	280 58.4	248 57.5	87.8
4	♀	72	61 45.0	—	100 48.4	—	100 48.4	93.0
5	♂	22	88 44.9	58 48.6	112 49.5	132 50.7	122 50.1	89.6
6	♀	42	87 38.6	90 38.6	104 41.8	128 42.8	116 42.3	91.3
7	♀	36	54 43.1	55 42.8	100 47.5	90 48.5	95 48.0	86.9
8	♂	27	37 41.8	44 45.8	95 47.0	108 48.5	102 47.8	87.4
9	♂	53	57 38.6	61 41.8	133 43.6	128 45.0	131 44.3	87.4
10	♀	61	80 40.2	—	97 43.0	103 43.0	100 43.0	93.5
Mean ± S.E.M.			70 ± 10 43.7 ± 1.4					Mean ± S.E.M. 89.1 ± 0.9

ing position as of the supine values was calculated as follows: $COP_{supine} \times 100 / COP_{standing}$. Plasma volume during exercise as % of the resting value was calculated in the same way.

A Postural studies after salt depletion

A sodium free diet (10 mEq daily) was given for 3 days. The third day 100 mg of chlorthalidone (Hygroton®) was administered perorally in the morning. The experiments were carried out on the fourth day between 8 and 10 a.m. After 45 min in the supine position the patient was assisted to a standing position and blood samples were obtained at intervals as shown in Table I.

B Exercise studies

Seven individuals carried out eight experiments on a bicycle ergometer. After a resting period of 20 min on the bicycle saddle the first blood sample was drawn, the other samples being taken at intervals as indicated in Table II. The exercise performed for 30 min was moderately heavy (600–800 kg m/min). The pulse rate at the conclusion of exercise was between 120–140/min. The experiment took place between 8 and 17 a.m.

RESULTS

A Postural studies after salt depletion (Table I)

The mean supine plasma renin activity was 70 ng angiotensin/10 ml plasma/4 h incubation ± 10 (S.E.M.) which is significantly higher than on

normal diet (normals on liberal salt ingestion, mean 15 ng ± 2 (12) $p < 0.001$). Mean supine COP was 43.7 cm H₂O ± 1.4 which is significantly higher than on normal diet (normals on liberal salt ingestion mean 34.8 cm H₂O ± 0.6 (12) $p < 0.001$). The increase in plasma renin activity from the supine to erect position is significant in all cases (the 95% confidence limit of the method is exceeded). After 20 min of standing a plateau is reached in all experiments. This increment is preceded by a rise in COP in all cases. After 20 min a plateau is attained in COP also. These findings are the same as in postural changes on normal salt ingestion (11). Mean plasma volume in the erect position as % of the supine value is 89.1 which is significantly less than in corresponding investigations on normal sodium intake (normals 84.6% ± 0.9 (17) $p < 0.001$).

Fig 1 shows as abscissa the increase in CC and as ordinate the increase in PRA from supine position to 20 and 30 min of erect position. correlation between ΔCOP and $\Delta PRA = 1$. The regression line is $y = 13.7x - 15.2$ $r = 0.9$

Table II Exercise

0=sample after 70 min of resting position on the bicycle 5 10 20 and 30= min after start of exercise Mean COP during exercise is obtained as average of 20 and 30 min values Work load is indicated in kg m/min The two experiments performed in case 3 are indicated as 3a and 3b

Case no	Sex	Age (y)	PRA COP	PRA ₅ COP ₅	PRA ₁₀ COP ₁₀	PRA ₂₀ COP	PRA ₃₀ COP ₃₀	Work load	Mean COP during exercise	Plasma volume during exercise as % of resting plasma volume
1	o	24	21 39.9	40 44.3	— 43.0	— —	43 44.2	900	44.2	90.3
2	♀	22	43 38.5	39 40.1	64 41.5	69 44.0	71 43.3	600	43.7	88.1
3a	♂	28	40 42.6	36 44.0	39 44.7	58 43.7	54 44.8	900	44.3	96.2
3b	♂	28	39 45.8	68 45.1	— —	60 44.6	— —	700	44.6	107.7
4	o	30	40 45.9	40 46.6	40 47.8	76 46.5	76 46.7	700	46.6	98.3
5	♂	34	30 42.3	50 46.0	56 46.7	— 46.5	60 47.5	600	47.0	90.0
6	♂	23	48 42.6	51 42.6	— —	79 41.6	85 43.0	700	42.3	100.7
7	♂	40	8 41.9	12 44.0	— —	28 44.5	37 45.5	600	45.0	93.1
										Mean \pm S.E.M. 95.0 \pm 1.9

$p < 0.001$ The corresponding regression line for normals on liberal salt intake (11) is drawn in Fig 1 as well The slope of the line of salt depleted persons is significantly steeper ($p < 0.001$)

B Exercise studies (Table II)

The increase in PRA from resting to exercise values is significant in all cases A plateau is already reached after 5 min of exercise in cases 1 3b and 5 and is found in all cases after 20 min Though an attempt was made to maintain equally heavy exercise for all which seems to have been achieved (small differences in pulse rate at the end of exercise) the COP rise varied from experiment to experiment In some of them it did not rise at all (cases 3b 4 and 6) The greatest increase corresponded to an exercising plasma volume of 88.1 % of resting plasma volume Mean exercising plasma volume as of "resting" plasma volume is 95.0 which is significantly less than in the corresponding postural study ($p < 0.001$) In all experiments in which COP did rise a plateau was attained in the blood samples drawn after 5 min of exercise except in case 2

Fig 2 shows the increase in COP from resting values to values after 20 and 30 min of exercise as abscissa and the increase in PRA in corresponding samples as ordinate The correlation $\Delta\text{COP}-\Delta\text{PRA}$ is not significant The correlation line is calculated by the method of least squares $y = -1.9x + 34.5$ $r = -0.31$ ($0.30 < p < 0.40$)

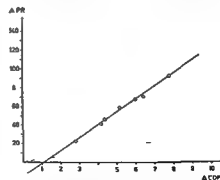


Fig 1 Postural experiments Normal individuals after 3 days of salt depletion Correlation between increase in plasma renin activity (ΔPRA) and increase in plasma colloid osmotic pressure (ΔCOP) from resting values after 0 and 30 min of standing The dashed line is a demonstration of the regression line of normal individuals on liberal salt intake

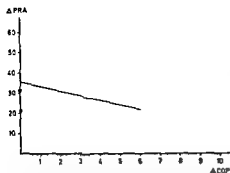


Fig 2 Correlation between increase in plasma renin activity (Δ PRA) and increase in plasma colloid osmotic pressure (Δ COP) from resting values to values after 20 and 30 min of ergometer exercise

DISCUSSION

In the postural studies carried out after salt depletion a linear correlation between Δ COP and Δ PRA was found but the slope of the regression line was steeper which may be interpreted as a sensibilization of the stimulus of renin release caused by the sodium depleted state (PRA increase per unit COP increase is greater). A similar sensibilization has previously been reported by Bunag et al (1) who demonstrated a potentiated renin response to constriction of a renal artery in chronic salt depleted dogs. If such a sensibilization took place during the course of acute salt depletion the Δ COP- Δ PRA relationship would not be rectilinear but curvilinear. As there are no indications of this (11) it is conceivable that a chronic salt depletion (lasting some days) is necessary to produce this effect. There is a certain overlapping between the range of supine PRA for salt depleted individuals (35–148 ng) and the corresponding range for individuals on liberal salt ingestion (0–36 ng) but the relationship Δ COP- Δ PRA follows different regression lines. This suggests that the normal range of supine PRA is not greatly influenced by various salt ingestion.

In the exercise studies no correlation was found between Δ COP and Δ PRA suggesting a different mechanism of stimulation. Castenfors (3) has reported that the renin increase during exercise in rats could be prevented by ganglion blocking agents therefore concluding that the autonomic nervous system is of importance to renin release. He points out the possibility of a direct sympathetic stimulation of the juxtaglomerular apparatus

because treatment with dihydralazine minimizing the renal vasoconstriction did not influence the renin response. Bunag et al (1) have found the sympathetic system of significance for renin release in bleeding dogs as ganglion blocking and local anesthesia of the kidney nerves inhibited a rise in renin. Other investigators have found similar evidence for a role of the sympathetic system in renin stimulation (5, 14, 15). On the other hand we know that the homotransplanted human kidney reacts to salt depletion and changes in posture by renin increase as in normal individuals (6, 9). Vander and Luciano (13) have also found that renin increase in acute sodium depleted dogs may take place although the sympathetic nervous system has been thoroughly blocked by surgical denervation of the kidney. Local anesthesia of the kidney hilus and alpha and beta blocking agents. The author mentions the possibility of an unknown humoral factor as stimulator in this situation. The quoted studies point to the presence of a neural and a non neural (humoral?) stimulating mechanism of the renin system. Our investigations are compatible with this concept. It is suggested that Vander's humoral factor may be some function of hemoconcentration (viscosity increase, COP increase or both).

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ABNORMAL GROWTH HORMONE RESPONSE TO INGESTION OF GLUCOSE IN JUVENILE DIABETICS

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Abstract. Using a chromatographic radioimmuno logical method serum growth hormone has been determined in thirteen controls and six newly diagnosed untreated juvenile diabetics during a six hour oral glucose tolerance test under strictly standardized conditions. The controls had a mean peak value of serum growth hormone five hours after glucose ingestion while the juvenile diabetics exhibited an early hyperresponse in serum growth hormone with a mean peak value 2 1/2 hours after glucose. No difference could be demonstrated between fasting serum growth hormone in the two groups. These results are discussed in the light of the hypothesis of Rabinowitz and Zierler and of Young.

The role of growth hormone in intermediary metabolism and especially in the homeostasis of blood sugar has been well known for many years on the basis of experiments in animals and studies in human subjects (6-11, 15). It has been suggested that an abnormality of growth hormone secretion may be of importance in the pathogenesis of human diabetes mellitus (37). However serum growth hormone has not been shown to be abnormal in diabetic patients.

Since the advent of the radioimmuno logical principle for plasma hormone determination our knowledge of the dynamics of growth hormone has been very much extended. Roth et al. (25) have described fluctuations in plasma growth hormone during oral glucose loading: a primary suppression 1-3 hours after glucose followed by an increase in the plasma concentration of growth hormone 4-5 hours after glucose. They also showed that insulin hypoglycaemia or a drop in intracellular glucose after administration of 2

deoxy glucose is accompanied by a significant rise in plasma growth hormone.

Plasma growth hormone has been found to be normal in fasting diabetics (8, 13, 36). The possibility exists, however, that the factors and mechanisms regulating growth hormone secretion are altered in diabetics. This problem has been studied extensively in maturity-onset diabetes but no significant abnormality has been found (13, 34) with the exception of the growth hormone response to arginine (17, 30) which is found to be depressed in female patients. The clinical picture and the biochemical pattern (including plasma insulin) of juvenile diabetes differs in many respects from that of maturity-onset diabetes. However very little attention has been paid to plasma growth hormone in this condition.

The following is a report of the results of serum growth hormone determinations using a chromatographic radioimmuno logical method in a group of untreated newly diagnosed juvenile diabetics. Growth hormone was determined in the fasting state as well as during an oral glucose load with 100 g of glucose.

MATERIAL

Diabetic subjects

Six patients with newly diagnosed untreated diabetes mellitus of the juvenile type admitted to the Second University Clinic of Internal Medicine, Kommunehospitalet, Aarhus. The age of the patients ranged from 10 to 33 years with a mean age of 19 years. Five of the six patients had intermittent or permanent ketonuria. The fasting blood sugar on the day of examination ranged between 147 and 301 mg per 100 ml with a mean fasting blood sugar value of 207 mg per 100 ml.

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the twelve subjects examined after repeated infusions or venous catheterization. The average fasting serum growth hormone level in the small group of juvenile diabetics stated here is in agreement with other studies and that juvenile diabetics have a normal fasting serum growth hormone level (1, 20).

However, during a six hour glucose tolerance test an early hyperresponse in growth hormone secretion was observed in juvenile diabetics. This abnormality has not been described before.

Parker et al. (20) measured the growth hormone response to a glucose load in 14 diabetic children and 15 normals of the same age. They concluded that there was no statistically significant difference between the growth hormone response to glucose in diabetic and normal children. However, scrutinizing their table it does seem as if there is a tendency to an earlier rise and to a broadening of the peak in the diabetics. The severity of the diabetes in the individual cases seems to have varied greatly, as the admission blood sugar ranged from 83 to 770 mg per 100 ml. This pronounced scatter may explain the lack of a statistically significant difference.

The study of Baker et al. (1) on the mechanism of partial remission in juvenile diabetics contains plasma growth hormone values obtained during an oral glucose load in ten untreated juvenile diabetics and ten normal children. In non-diabetics they found in agreement with all other investigators a growth hormone peak about 4 to 5 hours after glucose. Surprisingly the plasma growth hormone curves after glucose in the diabetics showed no significant change at any time during the test.

The mean curve from our six cases shows a peak at $2\frac{1}{2}$ hours after glucose. This peak is significantly higher than the lowest value found during the glucose load. The early high value found in a patient (J. N.) already $\frac{1}{4}$ hour after glucose ingestion might have been caused by sampling procedures as also in control no. 9 as discussed above.

The abnormal growth hormone response to glucose in juvenile diabetics demonstrated in the present study may be compared with the results obtained recently by Prange and Johansen (21) in this laboratory. Studying blood sugar, NEFA, serum insulin, serum growth hormone and serum glucagon every 30 minutes for a 24 hour period

of daily life they observed that growth hormone peaks occurred more often and that they were higher in juvenile diabetics than in non-diabetics. Some of the peaks in diabetics occurred just after meals and without relation to blood glucose or NEFA.

The late rise in plasma growth hormone in normal individuals is usually explained as a response to tissue starvation which results in a suppression of glucose uptake in the muscle and in the release of fatty acids from the deposits.

Glucose uptake in muscle is very low or zero in the fasting state and also during glucose loading in untreated juvenile diabetics as has been shown by Butterfield and Wichelow (3) and by Ørskov and Christensen (38). Assuming that the same is true as regards fat tissue, the other large metabolizing area in the body, there seems to be very little or nothing for growth hormone to suppress in juvenile diabetics.

It might be however that the early rise of growth hormone in juvenile diabetics observed in the present study is not of the same nature as that found in non-diabetics. Perhaps it should be compared to the unexplained plasma growth hormone peaks occurring now and then during a 24-hour period (8, 14, 18, 22, 29).

Rabinowitz and Zierler (23) have proposed a hypothesis to explain the pattern of plasma insulin and growth hormone in the fasting state and after food intake. They suggested a three-phase cycle: the first phase starting immediately after eating, the second phase two to four hours after eating, and the third later than four hours after eating. The first phase is characterized by a high plasma insulin and a low plasma growth hormone concentration; in the second phase the plasma insulin concentration is still high but plasma growth hormone is increasing; in the last part of the phase the third phase is dominated by a high plasma growth hormone and a low plasma insulin concentration. This model fits well with our concepts of carbohydrate, protein, and fat metabolism during and after a meal in the normal individual.

If in some condition, e.g. a genetic abnormality, the growth hormone response to glucose was changed towards an earlier peak, the proportion between the available amounts of insulin and growth hormone in the beginning of the second phase of Rabinowitz and Zierler

would obtain requiring a higher insulin production to assure normal metabolic events. Such a stress of the beta cells of the pancreas might finally result in an exhaustion of the pancreas leading to diabetes mellitus.

It is well known that no rise in plasma insulin occurs in classical juvenile diabetes after administration of glucose. This fact together with the early growth hormone peak observed in the present study may be explained as the end result of the hypothetical series of events described above and thus could fit into Young's theory about the role of growth hormone in the pathogenesis of diabetes mellitus. For this explanation to be acceptable prediabetic subjects should have the same early growth hormone peak but the insulin response to glucose should be augmented.

Immunologically detectable plasma insulin was determined during oral glucose loading in prediabetic subjects by several authors. The results obtained in these studies are however conflicting. Grodsky et al (10) found a normal insulin response in prediabetics. Ricketts et al (24) found significantly elevated plasma insulin one and two hours after glucose while Colwell and Lein (4) and Shima and Foa (27) found a tendency to a delayed hyperresponse in plasma insulin after oral glucose. Plasma growth hormone results obtained during oral glucose loading in prediabetic subjects are also controversial. To our knowledge only three investigations exist two of which are published in abstract form only (28-31) while the third deals with the growth hormone response to glucose in a family with heavy diabetic disposition (16). Unger et al (31) found a significantly higher plasma growth hormone response 4 and 5 hours after the glucose in prediabetics than in the controls. Levine and Recant (16) found an identical growth hormone pattern in a prediabetic group and a control group. In a recent publication Sonksen et al (28) described the growth hormone pattern in 18 male prediabetics after an oral glucose loading. They found the usual late rise 4 to 5 hours after the glucose in both the prediabetic and control group but seven of the 18 prediabetics exhibited also an early rise 15 to 45 minutes after the glucose compared to three of the 21 controls and the plasma growth hormone values obtained 15, 30, 45 and 60 min after glucose were significantly elevated in the prediabetic group.

In a study of the carbohydrate metabolism of patients with Turner's syndrome (18) we have found an abnormal glucose tolerance in six out of ten cases. In four of these cases serum insulin and serum growth hormone were studied after a glucose load. These patients with chemical diabetes showed an early peak in serum growth hormone. Serum insulin rises as in normal persons but remains high for several hours.

Another indication of an abnormality of growth hormone secretion in the prediabetic phase appears from a recent publication by Boden et al (2). They reported a slightly increased fasting plasma growth hormone as well as a hyperresponse to tolbutamide in genetically prediabetic patients.

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INFLUENCE OF GASTRECTOMY ON LEUCOCYTES IN BLOOD DURING EXERCISE IN TUBERCULOUS PATIENTS

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Abstract The leucocyte count of capillary finger blood determined at rest and during a 30 min exercise period in 13 gastrectomized and 9 non-gastrectomized patients with pulmonary tuberculosis is reported.

The increase with exercise of polynuclear and of total number of leucocytes was less ($p < 0.05$) in the group of gastrectomized patients. No relationships of significance could be observed between different absorption tests and the increase of leucocytes in blood with exercise in this material.

The number of leucocytes in blood at rest and during exercise was correlated ($p < 0.05$) with the concentration of gammaglobulins in blood and the tuberculin reactivity of the patients: those with weak reactivity and low gammaglobulin concentrations and low leucocyte values.

An increased incidence of tuberculosis in patients with gastrectomy has been observed in repeated studies (2, 3, 7, 16, 18). This has been attributed to the nutritional state of these patients since a correlation between tuberculosis and poor nutrition has been found (10).

The mechanism for this increased morbidity with nutritional deficiency is not known but some animal and clinical experiments suggest that an insufficient supply of proteins (14) or amino acids (11, 15, 22) and vitamins A and C (9, 11, 15) could be a causal factor. It has been suggested that patients suffering from gastric insufficiency have an unpaired cellular defence expressed as absence of leucocytosis in febrile infections (8).

As the leucocytes are part of the body's defence mechanism against infection it was considered appropriate to investigate whether or not a relationship between nutritional factors and amount of leucocytes in blood could be observed.

The amount of leucocytes in blood at rest, however, is not representative of the total number

of leucocytes in the body. During stress as after adrenalin injection (17) or during exercise (1) high leucocyte counts in blood have been recorded which has been attributed to a redistribution of leucocytes from other parts of the body. The amount of leucocytes in blood during a standardized exercise test might therefore be a better variable to study than the amount of leucocytes at rest.

MATERIAL

The patients in this study were all included in a larger investigation (10) in which the material is described in greater detail and more clinical data are given. All patients were hospitalized because of their pulmonary tuberculosis. One group (OP) consisted of 13 patients with previous gastrectomy (one B I, twelve B II); the other group (NO OP) of nine patients with only pulmonary tuberculosis.

The mean extent of tuberculosis was the same in both groups. Patients with cardiovascular diseases or abnormal ECGs at rest or during an exercise test were not accepted. Furthermore, only patients who after the preliminary exercise test were considered fit for a 30 min exercise period were included in the two groups.

METHODS

Apart from recording of routine clinical data such as tuberculous reactivity, extent of tuberculosis, bacillary occurrence and resistance, some special malabsorption tests were also performed (for details see (10)). The following nutritional variables were recorded: serum folic acid activity, vitamin A serum concentration and absorption, radio-B absorption, vitamin B₁₂ concentration, serum iron concentration and absorption, serum proteins and serum cholesterol.

The first work test consisted of a standardized exercise test (10) with recording of heart rate, electrocardiogram

Table 1 Some data obtained at rest or during two exercise tests in 13 gastrectomized and 9 non gastrectomized patients with lung tuberculosis

Mean values and standard errors of the means are given and the significance of the differences between the groups

	I Gastrectomized	II Non gastrectomized	Difference I II	P level
Age y	57.1 \pm 2.0	48.0 \pm 2.2	8.9	<0.05
1st test				
W ₅₀ kpm/min	565 \pm 42	672 \pm 47	-107	n.s.
W _m kpm/min	547 \pm 45	733 \pm 41	-189	<0.05
2nd test				
Work load kpm/min	388 \pm 35	555 \pm 38	-167	<0.05
Heart rate beats/min	144 \pm 4.7	146 \pm 4.1	-2	n.s.
Work load W _m	0.72 \pm 0.02	0.75 \pm 0.0*	-0.03	n.s.
Leucocytes mm ³ At rest	5985 \pm 467	5589 \pm 378	396	n.s.
Leucocytes mm ³ Max. value during exercise	9119 \pm 742	9689 \pm 742	-570	n.s.
Leucocytes mm ³ After 30 min exercise final value	7965 \pm 851	9*33 \pm 738	-1268	n.s.
Leucocytes increase with exercise final to resting value	40.0 \pm 9.0	67.7 \pm 5.9	-27.7	<0.05

and respiratory rate during stepwise increased work loads on a bicycle ergometer (12). As most subjects were exhausted at heart rates well below 170 beats/min, the work load at heart rate (W_h) was determined instead of W_m as well as the work load at maximal working intensity (W_m) (73).

The second work test consisted of a 30 min exercise period in sitting position at a work load (W) of about 1/2 of W_m. Both W_h and heart rate after 30 min exercise were used as variables reflecting the relative work load. The leucocytes in capillary finger blood were determined in duplicate III rest in supine position before the exercise and thereafter every 5 min during the exercise period.

For leucocyte counts standard methods were used as described by Ahlborg (1). Detailed differential count was not performed, but polynuclear and mononuclear corpuscles were determined separately. All blood samples were taken and prepared by the same nurse. She also performed all the cell counts.

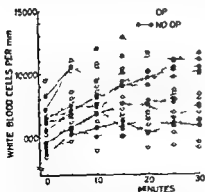


Fig. 1 Leucocyte counts in blood at rest and during sitting exercise in 13 gastrectomized (OP) and 9 non-gastrectomized (NO OP) patients with pulmonary tuberculosis.

In seven patients the leucocyte count was simultaneously determined in arterial blood samples. There was no significant difference between the arterial and the capillary blood samples ($p > 0.05$). Considering these two samples as double determinations the standard deviation for a single determination for leucocytes in blood at rest or during exercise was $\pm 9.5\%$.

Statistical calculations were made according to Snedecor (21). The following probability (P) levels of significance were used: $p < 0.001$ highly significant, $p < 0.01$ significant and $p < 0.05$ probably significant. Regression and correlation analyses were performed by the method of least squares on an IBM 360 computer.

RESULTS

Some data from the gastrectomized group and the control group are shown in Table I. On the average the gastrectomized patients were older

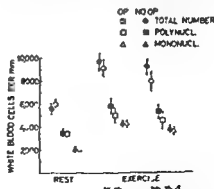


Fig. 2 Mean values \pm standard error of the means for leucocytes at rest and during exercise. Symbols as in Fig. 1.

and performed a lower maximal work load in the first test and a lower work load during the second test when the leucocytes were counted. Age (range 37-70 y) work load (200-750 kpm/min) heart rate after 30 min exercise (123-174 beats/min) maximal work load (300-900 kpm/min) and the work load in per cent of the maximal work load (58-88) did not significantly influence the leucocyte counts during exercise in this material. As was expected the 30 min work load the heart rate at this load and the maximal work load decreased with rising age.

The individual values for leucocytes at rest and during exercise are given in Fig 1. The average values at rest and peak and final values during exercise of total number of leucocytes, polynuclear leucocytes are given in Fig 2. There was no difference in absolute numbers between the two groups.

Peak values for total leucocytes were reached after 25 min exercise in the control group and after 19 min in the gastrectomized group, the difference not being significant. The relative increases of leucocytes with exercise are given in Fig 3. The percentage increase in both total number of leucocytes and in polynuclear leucocytes from rest to the end of the work test was significantly less in the gastrectomized group ($p < 0.05$).

When the absolute values of the leucocyte counts at rest and during exercise as well as the relative increases with exercise were related to the clinical data, there were no relationships of even

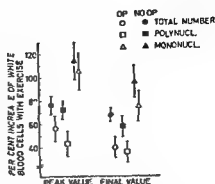


Fig 3 Mean values for increase of leucocytes during exercise. Symbols as in Figs 1 and 2.

probable significance with the nutritional and absorption tests. The only data that were related to the leucocyte counts were the tuberculin reactivity and the amount of gammaglobulin in blood. These relationships are given in Table II. The patients with weak tuberculin reactions had lower leucocyte counts at rest and during exercise than those with marked tuberculin reactions. Patients with low proportion of gammaglobulin in blood had lower leucocyte counts during exercise than those with high gammaglobulin values.

DISCUSSION

Active forms of tuberculosis like other infections result in a more or less pronounced rise of leucocytes. This is interpreted as a sign of an intact defence mechanism of the body. It has been sug-

Table II Relationships between some clinical data and different leucocyte variables in 22 patients with pulmonary tuberculosis

The significance of the regression coefficients (*b*le) is given

Independent variable (x)	Dependent variable (y)	<i>b</i> s
Tuberculin reaction (n=22)	Leucocytes at rest	-2.90
	Leucocytes max. value during exercise	-2.23
	Leucocytes after 30 min exercise	-2.53
	Polynuclear leucocytes max. value during exercise	-2.71
	Polynuclear leucocytes after 30 min exercise	-2.55
Gammaglobulin in blood mg/100 ml (n=15)	Leucocytes max. value during exercise	2.31
	Leucocytes after 30 min exercise	2.90
	Increase of leucocytes from rest to max. value during exercise	2.54
	Polynuclear leucocytes max. value during exercise	2.76
	Polynuclear leucocytes after 30 min exercise	2.94

Tuberculin reaction classified as follows: 1) negative to 1 mg of OT; 2) positive to 1 mg of OT; 3) positive to 0.1 mg or more of OT; 4) positive to 0.01 mg or more of OT.

gested (8) that febrile infections in patients with gastric insufficiency are not infrequently associated with leucopenia, or at least the absence of leucocytosis due to an impaired cellular defence.

The gastrectomized tuberculous patients in the present study did not increase their leucocytes during the extended physical stress as much as the non-gastrectomized. This is in agreement with the earlier suggestion (8). It is not likely that this difference between the groups was caused by the lower mean work load and higher mean age in the gastrectomized group as no effect of age per se on the increase of leucocytes with exercise could be observed within the present material and no effect of the relative or absolute work load.

The observed increase of leucocytes in blood by about 50% after 30 min of exercise is far above the expected 10% of hemoconcentration due to the exercise (5) and can thus not be ascribed to this hemoconcentration alone. In a group of young normal men the increase of leucocytes with exercise was found to be best correlated to work time but also significantly to absolute and relative work load (1). The increase of total number of leucocytes with exercise was in the present study of elderly patients similar to the increase in the group of young men although the absolute work load was less. As the heart rates were similar however the relative work load was on the average probably higher for the elderly patients with an expected higher maximal heart rate. In the present study the number of mononuclear leucocytes increased more with exercise than the number of polynuclear. In the group of normal young men (1) the opposite was the case but whether this difference should be attributed to the disease cannot be evaluated at present. However a lymphocytic phase of leucocytosis with exercise has previously been described, preceding a neutrophilic and a toxic phase (4, 13).

Though there is a correlation between tuberculosis and postgastrectomy state this does not necessarily mean that the low leucocyte response to the infection is impaired by malnutrition. In the present study no relationship between the amount of leucocytes and the nutritional or absorption test used could be demonstrated.

Folate deficiency with hypersegmented polymorphs has been found in tuberculous patients in England (19) but not in Sweden (6). However

folate deficiency is frequently found in gastrectomized tuberculous patients (6) and in this study five of the gastrectomized patients had subnormal folate values versus one patient in the non-operated group. Evidently this deficiency does not seem to influence the amount of leucocytes. The lack of leucocyte response in physical stress and the impaired nutritional state in gastrectomized patients may be two independent factors in the malabsorption syndrome. However it has to be emphasized that the number of patients was small and the list of nutritional and absorption tests used in this study was not complete. There may be other variables to investigate e.g. amino acids that may be better related to the general protein synthesis. The correlation found between low gammaglobulins and low leucocyte counts may also be attributed to a low protein synthesis in general. The role of lymphocytes for gamma globulin production is well known but a correlation between low mononuclear counts and gammaglobulin could not be found in the present investigation.

The patients with low leucocyte counts also had low tuberculin reactivity. The simultaneous lack of both these diagnostic signs in patients with active extended tuberculosis with bacilli may have to be considered in the single case.

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THE EFFECT OF MORPHINE ON ARTERIAL BLOOD GASES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract The arterial blood gas state was examined before and after an intramuscular injection of 1 cg morphine chloride in ten patients with uncomplicated acute myocardial infarction. Before morphine the CO_2 tension was normal, whereas the O_2 tension was definitely but moderately reduced. After morphine there was a slight tendency to an increase in the CO_2 tension associated with an even smaller further decrease in the O_2 tension. Two patients with clinical signs of moderate pulmonary congestion showed the greatest blood gas disturbances both before and after morphine. The data gave no evidence that morphine caused further impairment of tissue oxygenation.

Recently there has been some discussion about the place of morphine in the treatment of acute myocardial infarction but relevant clinical studies are few. Thomas et al. (8) studied the hemodynamic effects of intravenous injection of morphine in patients with acute myocardial infarction and concluded that the circulatory response to morphine was very variable and not predictable. However, they found no consistent changes in the arterial blood gases.

Kirby and McNicol (1) studied patients with acute myocardial infarction and found a high mortality rate in patients with uncompensated metabolic acidosis. They suggested that care be taken with any procedure such as the administration of morphine that might interfere with the compensatory pulmonary hyperventilation.

The need for further investigation in this field has led to the present study on the effects of intramuscular injection of 1 cg of morphine chloride on the arterial blood gases in patients with uncomplicated acute myocardial infarction.

MATERIAL

Ten patients, nine men and one woman, aged 40 to 68 years (mean 57.7) with acute myocardial infarction were

studied 1 to 48 hours after the onset of symptoms. All were in normal sinus rhythm, none had arterial hypotension, severe pain or Cheyne-Stokes respiration. Two patients had persistent basal pulmonary crepitations, none of the other patients showed any evidence of pulmonary congestion. None of the patients had been given morphine or any medication known to influence the cardio-pulmonary function prior to the start of the study. The patients had no oxygen for at least two hours before the start of the study.

METHOD

The patients were studied in the supine position in bed with the head slightly raised breathing air. Arterial blood was withdrawn through a polyethylene catheter in the femoral artery inserted at least 15 min before the start of the study. After collection of two control samples taken 10 min apart 1 cg of morphine chloride was injected intramuscularly. Then in the course of the following 60 min a further six samples were withdrawn at 10 min intervals.

Throughout the study blood pressures were recorded by a sphygmomanometer on the right arm and notes on the patients' clinical condition were taken. The patients were not allowed to fall asleep.

The oxygen tension (PO_2) and the carbon dioxide tension (PCO_2) were measured by means of an Instrumentation Laboratory Inc. Gas Analyzer Model 113 and pH was measured on a Radiometer pH meter Model 27 and a Radiometer Micro Electrode Unit. The hemoglobin oxygen saturation (SO_2) was read from a Radiometer Blood Gas Calculator (6). The correctness of the readings was frequently checked by direct spectrophotometric determinations with a Beckman Spectrophotometer Model DU according to Rasmussen (5).

RESULTS

The results are shown in Fig. 1. Many of the patients showed considerable variations in the blood gases during the observation period both before and after the injection of morphine.

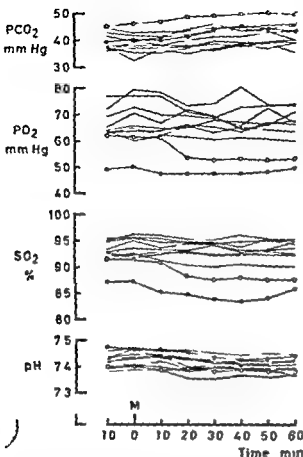


Fig 1 The effect of 1 cg morphine chloride i.m. on the arterial PCO_2 , PO_2 , SO_2 and pH in ten patients with acute myocardial infarction. M indicates injection of morphine. The curves marked by open and filled circles represent two patients with mild pulmonary congestion.

Before Morphine

Clinical

Some patients complained of slight chest pain but the majority were without symptoms. Two patients had some crepitations over the bases of the lungs posteriorly.

Blood pressure

One patient had a blood pressure of 215/140 and two others had moderate systolic hypertension. The rest of the patients had normal pressures.

Blood gases

The two patients with pulmonary congestion showed the most pronounced deviations from normal. When these two patients are excluded and the mean of the two sets of observations is

taken as representative for the single patient it appears that PCO_2 was within the normal range (35–45 mm Hg) while PO_2 was below the lower limit of normal for the relevant age groups (80–75 mm Hg) in all patients. SO_2 was at the lower limit of normal (95%) or below. However neither PO_2 nor SO_2 were markedly reduced, all values of PO_2 being higher than 60 mm Hg and of SO_2 above 91.0%. pH was generally in the high region of the normal range (7.35–7.45). The averages were PCO_2 38.5 mm Hg, PO_2 68.3 mm Hg, SO_2 93.9%, pH 7.426.

After Morphine

Clinical

In all but one patient morphine caused drowsiness and/or relief of pain—from 10 to 20 min after the injection. One patient complained of short lasting nausea 4 min after the injection of morphine but there was no accompanying change in blood pressure. In the two patients with crepitations over the bases of the lungs the auscultatory findings were not altered after the administration of morphine within the observation period.

Blood pressure

Of the three hypertensive patients two showed a moderate fall in systolic blood pressure; otherwise there were no changes in blood pressure.

Blood gases

The two patients with pulmonary congestion showed a clear tendency to further impairment of the blood gas picture, particularly with regard to PCO_2 and SO_2 . However in the most hypoxic of the two a marked increase in PCO_2 from 39.5 to 45.0 mm Hg was associated with a very small further decrease in PO_2 from 50 to 47.5 mm Hg. The other patient with pulmonary congestion showed a rise in PCO_2 from 46.0 mm Hg to 50.0 mm Hg and a fall in PO_2 from 61.5 mm Hg to 54.5 mm Hg. In these two patients SO_2 decreased from 87.2 to 83.4% and from 91.5 to 87.5% respectively. In the remaining patients who did not have pulmonary congestion there was a slight tendency to increase in PCO_2 and decrease in pH but no obvious changes in PO_2 and SO_2 . The maximal changes of the average figures were found 30 min after the injection for PO_2 and SO_2 and 50 min after the injection for PCO_2 and pH.

averages at these observation times being PCO₂ 0.8 mm Hg, PO₂ 67.1 mm Hg SO₂ 93.2%. pH 7.404

DISCUSSION

The data show that uncomplicated acute myocardial infarction is usually associated with a fairly normal PCO₂ and a clearly though moderately reduced PO₂ in arterial blood. This demonstrates that there is generally no major change in the alveolar ventilation but a definite disturbance of the alveolar/arterial gas exchange. This is in agreement with the observations of others (2, 3, 4, 8). McNicol et al (3) and Valentine et al (9) who studied the effect on PO₂ of 100% oxygen breathing claimed that the hypoxemia was mainly due to ventilation/perfusion disturbances and could only to a minor degree be ascribed to true anatomical shunting while MacKenzie et al (2) and Pain et al (4) using the same approach found reason to put more emphasis on the shunting.

The injection of morphine was followed by a slight increase in PCO₂ and an even smaller further decrease in PO₂. This indicates that there is a slight tendency to depression of the alveolar ventilation and that, if there is any change in the alveolar/arterial gas exchange at all it seems to be improvement rather than impairment. This may be correlated with on the one hand the central respiratory depressant effect of morphine and on the other possible hemodynamic effects of morphine.

The patients with pulmonary congestion showed more pronounced blood gas abnormalities than those without, both before and after morphine. The injection of morphine was followed by a clear increase in PCO₂ but in the patient with the most serious disturbances of the alveolar/arterial gas exchange the morphine induced decrease in the alveolar ventilation was not associated with any further impairment of the ventilation/perfusion disturbances.

As regards the oxygen transport to the tissues it is seen that morphine caused a definite decrease in the arterial SO₂ in the patients with pulmonary congestion whereas in the other patients there was only a slight tendency to SO₂ decrease. The SO₂ decrease was due partly to the fall in PO₂ and partly to the fall in pH. None of the patients studied showed any change in the base excess (7) after the injection of morphine. The slight decrease

in pH is therefore above all a consequence of the increase in PCO₂ and cannot be ascribed as might have been suspected to a lactic acidosis due to arterial hypoxemia and/or reduced peripheral perfusion.

Thus in these patients there was no evidence that the injection of morphine was followed by gross insufficiency of tissue oxygenation. However the possibility cannot be excluded that the lowered hemoglobin oxygen saturation before and especially after the injection of morphine may have had an unfavourable effect on the injured myocardium particularly in the patients with pulmonary congestion.

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THE EFFECT OF MORPHINE ON BLOOD PRESSURE AND CARDIAC OUTPUT IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract A double blind investigation of the hypotensive effect of morphine in patients with uncomplicated myocardial infarction has been performed in 20 patients. Ten were given an intramuscular injection of 1.5 cg morphine sulphate and ten an injection of a placebo. Blood pressure was recorded with the patient lying flat in bed, head up in an armchair position. A slight tendency to development of orthostatic hypotension was observed after the injection of morphine. Cardiac output was determined in five patients with uncomplicated myocardial infarction after the i.v. injection of 0.75 cg morphine sulphate. No marked fall in cardiac output was observed.

Morphine is one of the drugs most commonly used in the treatment of patients with acute myocardial infarction. Although it has been widely used for many years the circulatory effect of morphine has not been much studied in man. From the few investigations performed it appears as first observed by Drew et al in 1946 (2) that morphine enhances the tendency to develop orthostatic hypotension in the normal subject.

Investigations in patients with acute myocardial infarction are even more scarce. Thomas et al (6) have demonstrated that severe hypotension may follow the intravenous administration of morphine in patients with acute myocardial infarction. This observation has led to investigations of the circulatory effects of alternative analgetics. In one investigation a fall in blood pressure was observed after the injection of pethidine (5) while heroin was found by the same investigators to have little effect on the cardiovascular system (4).

We feel that further investigations with regard to the hypotensive effects of morphine in patients with acute myocardial infarction are warranted. The results of such an investigation performed in

Medical Department VIII, Ullevaal Hospital on patients with uncomplicated acute myocardial infarction are given in this report.

MATERIAL

Patients with uncomplicated acute myocardial infarction were examined within 48 hours after the onset of symptoms. Excluded were patients with congestive failure or cardiogenic shock, patients who were not able to lie flat in bed and patients who did not tolerate an armchair position in bed for 15 min. All the patients had regular sinus rhythm.

METHODS

Investigations were not started until 6 hours had passed since the last injection of an analgetic. Oxygen was given by face mask at a flow of 5 l per min.

In the first investigation the orthostatic effect of morphine was investigated. Blood pressure was measured by the conventional technique in the right arm to the nearest 2 mm Hg.

The investigation was double blind. Ten patients received an injection of 1.5 cg morphine sulphate intramuscularly and another ten patients were given an injection of a placebo.

Blood pressure and pulse rate were first recorded with the patients lying flat in bed. The bed was then tilted until the patients were sitting in an armchair position and the blood pressure and pulse rate were subsequently recorded every 3 min for 15 min. The same procedure was repeated after an intramuscular injection of morphine and placebo respectively. The time schedule for the investigation appears in Figs 1-3.

In the second investigation the intra arterial pressure was measured with a Statham pressure transducer # 23 GB. Cardiac output was determined by dye dilution technique as previously described (3). After an initial rest of 30 min the patients were tilted 20° head up and cardiac output and blood pressure were registered. Morphine sulphate 0.75 cg was then slowly injected intravenously.

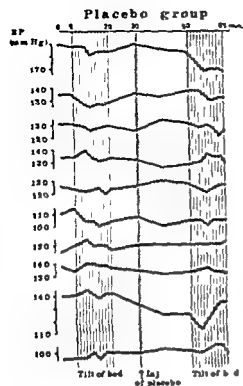


Fig. 1 Postural changes in systolic blood pressure before and after placebo

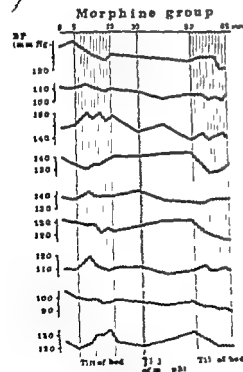


Fig. 2 Postural changes in systolic blood pressure before and after morphine

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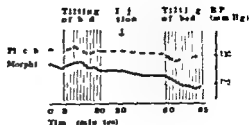


Fig. 3 Average blood pressures in morphine and placebo group

during 5 min. Subsequent recordings of cardiac output, intra-arterial blood pressure and pulse rate were performed 10 and 25 min after the start of the injection.

RESULTS

Orthostatic hypotension after morphine

The results from the double blind investigation with regard to the orthostatic hypotension after morphine appear from Figs 1-3. The systolic blood pressure recorded in the patients in the placebo group is shown in Fig. 1. No marked orthostatic hypotension was seen in any of the patients. The systolic blood pressure in the patients who were given morphine is illustrated in Fig. 2. No pronounced hypotension following the injection of morphine was registered. Neither bradycardia nor tachycardia was seen after the injection of morphine. None of the patients suffered nausea or vomiting. Average systolic blood pressures in the two patient groups are compared in Fig. 3. The average systolic blood pressure is higher in the placebo than in the morphine group. In both groups the systolic blood pressure fell during the investigation in the placebo group by an average of 4 mm Hg, in the morphine group by an average of 8 mm Hg.

The difference between the two groups with regard to orthostatic hypotension before and after morphine and placebo respectively was not statistically significant.

One patient in the control group had a labile hypertension with a marked fall in blood pressure during the second tilting of the bed. If this patient is excluded, no orthostatic fall in blood pressure was observed in the placebo group. The difference between the two groups with regard to orthostatic fall in blood pressure after the injection of morphine and placebo was then statistically significant ($0.02 < P < 0.01$).

Table I Cardiac output before and after injection of morphine

In each patient two or three measurements were taken before morphine injection one 10 min and one 25 min after the injection

Pat. no	CO before morphine (l min)	CO 10 min after morphine (l min)	CO 25 min after morphine (l min)
1	4.6	4.3	5.2
2	5.2	5.7	5.4
3	4.7	4.2	5.6
4	4.3	4.5	5.0
5	7.9	5.9	5.8

Cardiac output after injection of morphine

The results of the measurements of cardiac output are shown in Table I. No marked fall in cardiac output was observed in any of the patients. In one patient the systolic and diastolic blood pressure fell by 10 mm Hg following the injection of morphine, whereas the cardiac output remained unaltered. In the other four patients no change in blood pressure was registered.

DISCUSSION

The present investigation did not demonstrate any marked hypotensive effect of morphine in patients with uncomplicated acute myocardial infarction. A slight tendency to the development of orthostatic hypotension was however demonstrated. This may be of clinical importance during the transport of patients with acute myocardial infarction. If hypotension occurs as a result of the injection of morphine the foot end of the bed should be raised as advocated by Thomas et al. (6). During transport care must be taken not to carry the patient in a head up position after he has been given morphine.

Nausea and vomiting are frequently seen in patients with acute myocardial infarction and may usually be regarded as manifestations of the disease. Bradycardia and hypotension may be caused by the nausea. However, morphine in itself may provoke such symptoms although it was not observed in the present investigation. Bjerkelund et al. (1) have shown that perphenazin (Trifalon) provides protection against nausea and vomiting in patients with acute myocardial infarction. We

therefore routinely give perphenazin to patients who suffer nausea or vomiting after the injection of morphine.

CONCLUSION

From the present study of the circulatory effects of morphine in patients with uncomplicated acute myocardial infarction we are led to the conclusion that there is no strong justification for the advice that morphine should be substituted by other analgetics in the treatment of these patients.

However, one must be prepared to encounter the occasional hypotensive reaction caused by morphine. It can be controlled by elevating the patient's legs.

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CORONARY OCCLUSION TREATED WITH SMALL DOSES OF HEPARIN

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Abstract In common antithrombotic therapy the dosage of heparin is kept at a certain level based on prolongation of *in vitro* coagulation time. However, considerably lower doses have been reported to have effect in long term treatment of coronary heart disease. In order to investigate the effect of similar low doses in patients with acute coronary occlusion a double blind trial was undertaken, in which the death rates of patients treated with heparin for 24 days at a dosage of 10 000 units subcutaneously twice daily for the first 16 days and subsequently once daily was compared with a placebo-treated group.

Thirty two per cent of 103 heparin treated patients died compared with 41.3 of 109 in the placebo group. This difference is not statistically significant. Thrombo-embolic complications were however significantly less frequent in the treated group and it is concluded that the therapeutic experiment should be continued.

Antithrombotic treatment of coronary occlusion by heparin is usually based upon a considerable reduction of the blood's ability to coagulate *in vitro* and the doses needed vary from 5000 to 15 000 units given intravenously at intervals from 4 to 8 hours. When subcutaneous injections are preferred 25 000 units twice daily are considered sufficient.

Engelberg et al (3) in 1956 demonstrated that long term treatment of coronary heart disease with small doses of heparin—200 mg subcutaneously twice weekly—had a striking effect on the incidence of new coronary infarctions and sudden heart deaths. These observations were confirmed by Bottiger et al (2) in 1965 who made use of the same double blind technique.

In view of differences of opinion concerning the value of anticoagulant therapy in acute coronary occlusion it was considered important to investigate whether the course of the acute disease could be influenced by heparin in similar small doses.

The opportunity for undertaking such a study presented itself in Denmark in 1961. After the publication by Hilden et al (4) of the unsatisfactory results of anticoagulant therapy it was no longer thought unethical to withhold this treatment from patients.

METHODS

From November 1961 until June 1968 all patients with coronary occlusion under the age of 75 admitted to the Medical Department of the County Hospital Hobro were given either 10 000 units of heparin subcutaneously twice daily for 16 days followed by 10 000 units daily for 8 days—or corresponding volumes of placebo.

The trial was carried out according to the double blind principle. Heparin and placebo preparations were supplied by Leo Pharmaceutical Products, Copenhagen, in uniform packages each containing four capped vials with colourless fluid, half of which were solutions of heparin 10 000 units per ml. Each vial had a four-digit number referring to a secret code that was kept by a reliable non-medical assistant. At the beginning of the treatment a package was taken at random from a minor depot; the vial numbers were noted in the record and the patient's name was written on the package.

The diagnosis was made as soon as possible after the admission based upon the clinical details and ECG using 2 chest leads. According to the following course of the disease: repeated ECG, temperature reaction, ESR and G-O transaminase, the diagnosis was confirmed or rejected. Autopsy was performed in 11 cases of death (80%).

During part of the period coagulation time was determined in the patients at intervals of one day, one week and three weeks after the beginning of the treatment. Venous blood was taken in a clean and dry glass tube which was slightly tilted every minute and watched until the beginning of visible coagulation. The results were kept secret in the laboratory and only considered for statistical analysis at the end of the trial.

The treatment received by the individual patients was revealed at intervals when the series of patients had finished treatment, the issue was known and all prognostic criteria and complications had been recorded.

Table I Age distribution

Age	Heparin group No	Placebo group No
35-44	3	4
45-54	14	15
55-64	35	41
65-74	51	49

Table II Distribution of risk factors

	Heparin ()	Placebo ()
Previous coronary occlusion	17.5	23.0
Diabetes mellitus	9.7	6.4
Shock	5.8	5.5
Pulmonary oedema	13.6	8.3
Conduction disturbances	15.5	20.2
Congestive heart failure	20.4	23.8

Table III Death rate

Risk group	Heparin group		Placebo group		p
	Treated no	Dead (no) (%)	Treated no	Dead (no) (%)	
I	57	5 (9.6)	55	11 (20.0)	0.13
II	51	8 (15.9)	54	34 (63.0)	
Total	108	33 (32.0)	109	45 (41.3)	0.16

The patients were mobilized according to their individual clinical state by means of a 3-step graduation of bed rest after which they were taken out of bed. The average time spent in bed before getting up was 11.3 days in the survivors.

In other respects, treatment of the disease followed the traditional pattern and did not change much during the nearly seven year experimental period.

RESULTS

The diagnosis of coronary occlusion was made 263 times during the period. However in 25 cases the diagnosis eventually turned out to be incorrect. In nine of these patients the correction was made at the autopsy table. Thirteen patients had to be excluded as they died before therapy could be applied; five of these were diagnosed at autopsy. Patients who died less than one hour after the first injection are also included in this group and not considered in the study. Thirteen

patients were thought unfit for the trial for various reasons so the material used in the study was taken from 212 cases.

80.2% of the patients were males corresponding percentages for the heparin and placebo groups were 78.6 and 81.7. The age distribution is shown in Table I. The average ages were 62.6 and 62.9 years in the heparin and placebo groups respectively.

The distribution of common risk factors is shown in Table II. Diabetes mellitus and pulmonary oedema at admission were seen more frequently in patients treated with heparin while previous coronary occlusion, congestive heart failure and arrhythmias at the time of admission were more frequent in the placebo group.

The result of the therapy is shown in Table III. In risk group II one or more of the criteria mentioned in Table II were present in the patients. None of the differences between heparin and placebo groups in Table III are statistically significant.

Thrombo-embolic complications were recorded in 14 patients treated with heparin (13.6%) and in 28 patients in the placebo group (25.7%) this difference being significant ($p = 0.024$). In placebo patients these complications may have been more serious since 18 of them died compared with 11 deaths in the heparin group. The various types of complications recorded are shown in Table IV.

Table IV Thrombo-embolic complications

	Number of cases	
	Heparin	Placebo
Reinfarction	4	4
Plebitis	5	10
Pulmonary emboli		6
Systemic emboli	3	9
Parietal thrombi found at autopsy	5	14
Parietal thrombi clinically diagnosed	0	1

Table V Coagulation time

	No	1 day after admission		13 weeks after admission	
		Mean (sec)	SD (sec)	Mean ()	SD ()
Heparin	11	72.6	11.4	5.8	6.8
Placebo	23	209.9	12.6	11.8	-

A few haemorrhagic complications were seen in both groups none of them was severe. Coagulation times are shown in Table V. The differences between heparin and placebo treated patients are slight and not statistically significant.

DISCUSSION

According to prevailing concepts antithrombotic heparin therapy requires an augmentation of the coagulation time to 2-3 times the normal value. Some authors claim coagulation times of 20-30 min.

An analysis of the rationale of this procedure reveals some facts and some assumptions. Evidently determination of coagulation time is a practical method of measuring a single biological effect of heparin. The result depends in a predictable way on dose, method of application and duration after the injection.

It is also a fact that by injections especially using the intravenous route a considerable variation of concentration is obtained so that the effect at certain times must be a good deal greater than the therapeutic minimum.

It is assumed firstly that the antithrombotic effect of heparin is parallel to its ability to impede the coagulation of blood *in vitro* and secondly that the very strong effect sometimes met in injection therapy is on the whole harmless.

However the results of heparin therapy according to these principles did not show any advantage when compared with warfarin treatment (1) therefore it seemed justifiable to investigate a possible antithrombotic effect of heparin at a dosage lower than that previously used.

In the present series anticoagulation in the usual sense has not been intended nor has it been obtained as judged by the coagulation times found. Serious haemorrhagic complications were not expected and not observed. This suggests an advantage over common anticoagulation measures in which the efficacy of the therapy may be partially counterbalanced by its complications.

Studies on the effect of anticoagulation in coronary occlusion frequently do not include patients who died less than 48 hours after the beginning of the treatment even if this period is covered by heparin. However a great part of the fatal issues are seen during this first period and it is obvious that future investigations of anti-

thrombotic therapy should deal also with this critical period.

No definite conclusion can be made from this therapeutic experiment. Although the patients treated with heparin had a lower mortality rate the number of patients studied was too small to demonstrate that this difference in mortality did not occur by chance. The difference in thromboembolic complications may be a real effect of heparin in low dosage and suggests that the investigation should be continued.

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PQ INTERVAL DEPENDING ON POSTURE

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Abstract Three healthy persons with prolonged PQ duration in supine position are presented. The PQ is normal in standing position. From these cases and from the literature it is concluded that a prolonged PQ-interval in itself is no indication of heart disease and that in such cases a standing ECG should always be taken.

The PQ interval in the electrocardiograms is normally within the range of 0.12 to 0.22 sec. The duration is to some extent correlated to the heart rate. Abnormal PQ intervals are seen in patients with acute rheumatic fever, in acute myocardial infarction and in digitalis intoxication.

As abnormal PQ duration is most often seen in heart disease we find it important to stress that the PQ is seen to change significantly with posture in completely healthy persons.

New documentation of this fact is given by the history of three patients in whom the PQ interval was dependent on posture. A fourth case with arteriosclerotic heart disease with prolonged PQ not changing with posture is presented for comparison.

CASE REPORTS

Patient 1

A 40-year-old male who was first seen in our department at the age of 14 in 1961.

He had no heart complaints.

At auscultation a systolic murmur was heard at the left sternal border. The X ray of the chest was normal. The ECG showed a prolonged PQ-interval.

A right heart catheterization was done showing normal pressures and no intracardial shunts.

The patient was not seen until 1968 when he had to join the army. He was still without heart complaints. X ray of the chest was still normal.

At auscultation a systolic murmur grade I was heard at the left sternal border. It was of vibratory type.

The ECG was normal apart from a prolonged PQ-interval of 0.30 sec (Fig. 1). Standing ECG showed a PQ of 0.18 (Fig. 2). When the patient was asked to lie down

again the PQ after two min delay was again prolonged (0.30 sec). One mg atropine was given intravenously. Shortly after this the PQ fell to 0.18 sec. The effect subsided after some hours.

During exercise the PQ fell to 0.18 sec.

Conclusion

No organic heart disease. PQ-interval changes with posture.

Patient 2

A 39-year-old woman, who was admitted with fever. The diagnosis was pyelitis.

As the ECG showed prolonged and changing PQ interval, rheumatic fever and myocarditis were suspected. The examinations could not substantiate this suspicion. During treatment with antibiotics, the fever subsided.

The patient was then seen in our department. She had never fainted and had never had cardiac complaints.

At auscultation no murmurs were heard. Blood pressure was 100/70. The ECG is seen in Fig. 3. The PQ-interval was 0.28 sec. Standing ECG is shown in Fig. 4. PQ was 0.18 sec. The patient was asked to lie down again and after two min the PQ was 0.34 sec.

X ray of the chest was normal. AST 65-100 (normally below 200). ASH below 4000 (normally below 4000). L.E. cells negative.

Conclusion

No organic heart disease. PQ-interval changed with posture.

Patient 3

A 41-year-old male who was admitted for tonsillectomy. There was no family history of congenital heart disease. He had never had rheumatic fever or tuberculosis.

Since his early childhood he had had tonsillitis several times.

He had never had heart complaints and was a top-trained athlete. During six months he had had intermittent joint pains, but there had never been swelling of any joint.

He had never fainted.

At auscultation no murmur was heard. The second heart sound was split in the pulmonary area.

The X ray of the chest was normal.

The ECG (Fig. 5) showed right bundle branch block. There was wandering pacemaker and nodal rhythm. Stand

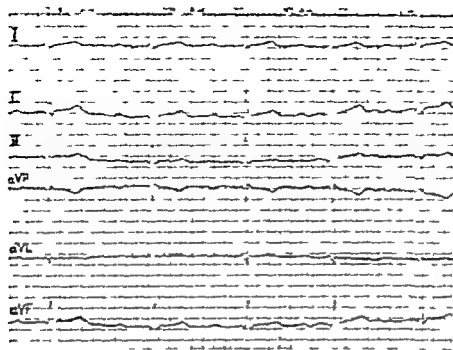


Fig. 1 Patient no. 1. ECG in supine position. PQ 0.33 sec. Heart rate 75. Paper speed 40 mm/sec.

ing ECG is shown in Fig. 6. The PQ was now normal. During and after exercise there was also normal sinus rhythm. The heart rate was 168.

ESR 4 mm in one hour Hb 14.5 g%, AST and ASH normal.

At death

No signs of rheumatic fever or rheumatic heart disease. Right bundle branch block and wandering pacemaker.

were found. In standing position the PQ was normal. His heart was considered to be normal.

Patient 4

A 72-year-old male with angina pectoris since 1941. During the last six years he had been subject to fainting fits. He complained of dyspnoea during exertion.

In 1946 he got a left-sided pneumonia.

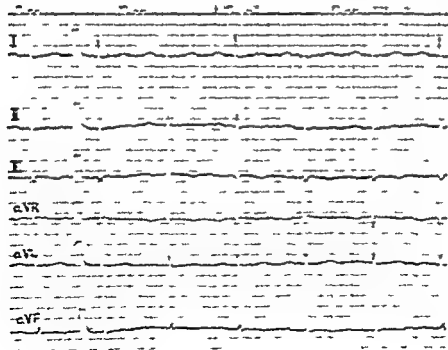


Fig. 2 Patient no. 4. ECG PQ 0.31 sec. Heart rate 94. Paper speed 40 mm/sec.

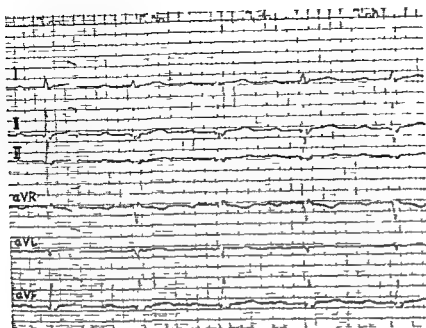


Fig 3 Patient no 2. ECG in supine position. PQ 0.28 sec. Heart rate 70 Paper speed 40 mm/sec.

He was now admitted because an A V block had been found

At auscultation no murmurs were heard. Blood pressure 150/60

No arterial pulsations could be felt in the feet.

The X ray showed an enlarged heart.

The ECG showed a left bundle branch block and a PQ-interval of 0.28 sec

The patient was put on digitalis treatment and there was no evidence of recent myocardial infarction.

Standing ECG and ECG after atropine still showed a PQ of 0.28 sec

Conclusion

Atherosclerotic heart disease. The prolonged PQ was not normalized in the standing position.

The faintings could have been Stokes-Adams attacks.

DISCUSSION

Atro-ventricular block of first second and third degree may be periodic or constant. All three types are seen in congenital heart disease either as a part of it or acquired, superimposed upon the deformity. The most common congenital defect with heart block is atrial septal defect (5). Block is also seen in digitalis intoxication, lues, rheumatic fever, diphtheria, chorea and in virus infections. The block in these cases is often of first or second degree but total A V block may be seen and was described in a 23 year-old soldier with German measles (3). The block disappeared when the infection subsided.

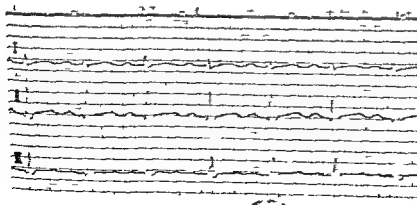


Fig 4 Patient no 2.5. ECG in standing position. PQ 0.18 sec. Heart rate 100 Paper speed 40 mm/sec.

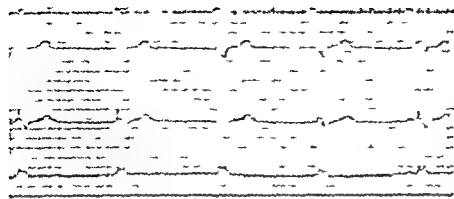


Fig. 5 Patient no. 3 ECG in supine position. Right bundle branch block. Normal PQ interval. Heart rate 60. Paper speed 25 mm/sec.

In acute myocardial infarction AV block is complete or complete transient or constant is frequently seen.

It is characteristic that, although the block may change during disease it has never been described as dependent upon the position of the patient.

Total AV block does not change with posture, not even in congenital block without other heart abnormality. In all these cases faintings may be seen.

The question now arises whether an abnormal PQ interval is seen in heart block and whether this PQ could be dependent upon the position of the patient. From the foregoing remarks, this should be suspected only in AV block of first and second degree. As seen from patient no. 3 postural normalization of the PQ may be expected in wandering pacemaker and may also be found in other disturbances of the AV conduction.

From the literature it is known that AV block is found in heart block. In a Finnish series of 650 athletes (2) a PQ interval of 0.22 to 0.40 sec. was found in 1 case. This abnormal data on

changed even during short time intervals but never became normal. On the other hand three of these athletes had a normal PQ when they were seen 20 years after they had given up athletics.

What posture means to the PQ has also been earlier described. Twents normal men, members of the Royal Air Force, showed a prolonged PQ in supine position (4) while sitting or standing ECG showed a normal PQ.

In this series the heart rate had no significant influence on the PQ interval. The men were examined on tilt tables and the PQ was normal in a position 90 degrees from the horizontal.

A follow-up study was performed on these men several years after the first examination. The

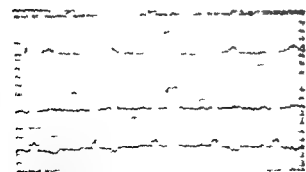


Fig. 6 Patient no. 3 Standing ECG. PQ interval 0.28 sec. Heart rate 80. Paper speed 25 mm/sec.

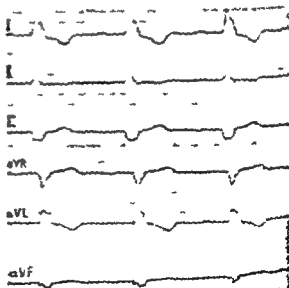


Fig. 7 Patient no. 4 ECG in supine position. Right bundle branch block. Normal PQ interval. Heart rate 60. Paper speed 25 mm/sec.

PQ was still prolonged and still changing with posture. No signs of organic heart disease were found.

Three healthy men were seen by Holmes and Weill (1) who found a prolonged PQ in supine position becoming normal in standing position. They found no correlation to heart rate.

The conclusion is that a prolonged PQ interval or otherwise abnormal PQ is not in itself an indication of organic heart disease.

If the history and all other examinations are normal a normal standing ECG speaks against organic heart disease.

The reason for a prolonged PQ is not known. In our patients the heart rate was higher in standing position and as atropine made the PQ normal a high vagal tone might be the explanation in these cases. Physical training may give a prolonged PQ and this could also be due to vagal tone but in many cases no definite explanation can be given.

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GASTRIC SECRETION OF ACID AND INTRINSIC FACTOR IN PATIENTS WITH HYPER AND HYPOTHYROIDISM

Gerhard Dotevall and Anders Wälan

From Medical Department II Sahlgrenska Hospital University of Göteborg Göteborg Sweden

Abstract Fifteen consecutive thyroid disease patients, ten with hyperthyroidism and five with hypothyroidism have been investigated as far as gastric secretion was concerned. Gastric secretion of acid and intrinsic factor was studied in the patients both basally and with the augmented histamine test. In eight patients with hyperthyroidism and all patients with hypothyroidism the gastric secretion of acid after histamine stimulation was significantly lowered. The secretion of intrinsic factor correlated with the secretion of acid and was significantly depressed in four patients with hyperthyroidism and three with hypothyroidism. Further studies showed pernicious anemia in two patients with hypothyroidism.

Disturbances of gastric secretion as related to thyroid gland dysfunction have been noted for many years. After the augmented histamine test normal or low acid output has been registered both in hyperthyroidism and hypothyroidism (3, 4, 11). An association with pernicious anemia has been described in both groups.

In an earlier publication we have noticed a high incidence of achlorhydria, hypochlorhydria and atrophic gastritis in patients with hyper and hypothyroidism (7). The present paper deals with the findings on gastric secretion of acid and intrinsic factor in these two conditions.

MATERIAL

Fifteen consecutive thyroid disease patients, four men and 11 women, entering either a medical or surgical ward were investigated (Table I). The mean age of the ten patients studied with hyperthyroidism was 42 years and of the five patients with hypothyroidism 59 years. The patients had a typical clinical history of hyper or hypothyroidism. In all cases except nos. 3, 4 and 10 PBI was taken and the highest value recorded (Table I). In these cases however BMR and/or RAI were taken. In cases 3 and 10 subtotal thyroidectomy was performed. Case 3 was later treated with radioactive iodine for recurrence. Case

4 was successfully treated with thyroxin blocking agents only. Five patients were euthyroid at the secretory test. Cases 2 and 9 were studied pre-operatively after having been euthyroid for 2-4 weeks. In cases 3, 4 and 7 the gastric secretory test was performed 3, 10 and 3 years respectively after they were made euthyroid.

METHODS

Gastric secretory studies were performed in the morning after at least 12 hours fasting. A nasogastric tube of 3 mm internal diameter was passed into the stomach. The patient was sitting in a semi-upright position. Continuous drawing of the stomach was carried out by suction at subatmospheric pressure of 50 mm Hg. Extra suction and injection of air was applied by means of a syringe. After removal of the residual secretion from the stomach a 60 min collection of basal secretion was made. Midway through this period 100 mg antazoline was given by intramuscular injection. At the end of the basal secretion, histamine HCl was given in doses giving maximal acid output (9). The volume of the gastric juice collected was measured and samples taken from each specimen for determination of HCl and intrinsic factor.

Hydrogen ion activity was determined in an automatic pH meter (Radiometer Copenhagen). Gastric juice was titrated with 1/10 NaOH until pH 7.0. Results are given as mEq/l/h.

The samples for intrinsic factor assay were neutralized with 10 N NaOH to pH 7.5, centrifuged to remove mucus and stored at -23°C before assay. Intrinsic factor activity was determined as described by Ardeaman and Chanarin (1) using Co vitamin B₁₂ as radioactive isotope. Intrinsic factor was expressed as the difference between total vitamin B₁₂ binding capacity and the vitamin B₁₂ binding capacity after complete blockage of intrinsic factor with excess of antibodies against intrinsic factor. One unit of intrinsic factor was defined as the specific intrinsic factor binding of 1 ng vitamin B₁₂.

Schilling test

The Schilling test was carried out as described by Grasbeck et al. (8) in some of the patients with achlorhydria or low acid output after histamine stimulation.

Table 1 Clinical and laboratory data on 15 patients studied with hyper or hypothyroidism

Pat. no	Age	Sex	Diagnosis	PBI ($\mu\text{g.}$)	Thyroid function at the secretory study	Remarks
1	60	♂	Hyperthyr	11.7	Hyperthyroid	
2	45	♀	Hyperthyr	9.9	Euthyroid	
3	56	♀	Hyperthyr	—	Euthyroid	BMR +56 RAI 7 h 64 Schilling test 26%
4	55	♂	Hyperthyr	—	Euthyroid	BMR +35% Schilling test 17
5	59	♀	Hyperthyr	12.1	Hyperthyroid	Serum Vitamin B ₁₂ 250 pg ml
6	50	♀	Hyperthyr	12.3	Hyperthyroid	
7	54	♀	Hyperthyr	13.8	Euthyroid	
8	26	♀	Hyperthyr	13.0	Hyperthyroid	
9	19	♀	Hyperthyr	9.9	Euthyroid	
10	76	♂	Hyperthyr	—	Hyperthyroid	BMR +35
11	67	♂	Hypothy	1.8	Hypothyroid	Schilling test without intrinsic factor 4% with intrinsic factor 11
12	52	♀	Hypothy	0.9	Hypothyroid	
13	64	♀	Hypothy	2.5	Hypothyroid	
14	56	♀	Hypothy	2.0	Hypothyroid	Schilling test without intrinsic factor 8%
15	45	♀	Hypothy	1.9	Hypothyroid	

RESULTS

Gastric secretion of acid

Basal secretion of acid was significantly lowered in patients with both hyper and hypothyroidism compared with controls (Figs 1 and 2). After histamine stimulation the acid output was within normal limits in two patients with hyperthyroidism (Figs 3 and 4). For all other patients the secretion of acid was significantly lowered.

Gastric secretion of intrinsic factor

Basal secretion of intrinsic factor was investigated in ten subjects with hyperthyroidism. In four patients the intrinsic factor secretion was lower than the lower range for hospital patients (2) (Fig. 5).

In all patients with hypothyroidism the basal secretion of intrinsic factor was low in three subjects < 100 units/h (Fig. 6).

After histamine stimulation the secretion of intrinsic factor was

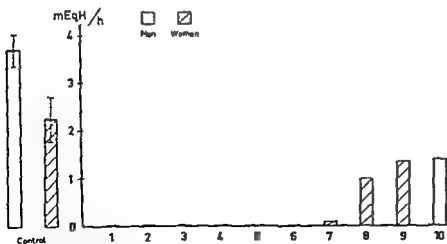


Fig. 1 Basal secretion of acid as mEqH/h in ten patients with hyperthyroidism. Mean and s.e. for controls according to Dotevall (6).

Table I Clinical and laboratory data on 15 patients studied with hyper or hypothyroidism

Pat no	Age	Sex	Diagnosis	PBI (μg)	Thyroid function at the secretory study	Remarks
1	60	♂	Hyperthyr	11.7	Hyperthyroid	
2	45	♀	Hyperthyr	9.9	Euthyroid	
3	56	♀	Hyperthyr	—	Euthyroid	BMR +56 RAI 7 h 64 Schilling test 26
4	55	♂	Hyperthyr	—	Euthyroid	BMR +35 Schilling test 17
5	59	♀	Hyperthyr	12.1	Hyperthyroid	Serum Vitamin B ₁₂ 250 pg/ml
6	20	♀	Hyperthyr	12.3	Hyperthyroid	
7	54	♀	Hyperthyr	13.8	Euthyroid	
8	26	♀	Hyperthyr	13.0	Hyperthyroid	
9	19	♀	Hyperthyr	9.9	Euthyroid	
10	26	—	Hyperthyr	—	Hyperthyroid	BMR +35
11	67	♂	Hypothy	1.8	Hypothyroid	Schilling test without intrinsic factor 4 with intrinsic factor 11 ^a
12	52	♀	Hypothy	0.9	Hypothyroid	
13	64	♀	Hypothy	2.5	Hypothyroid	
14	56	♀	Hypothy	2.0	Hypothyroid	Schilling test without intrinsic factor 8 ^a
15	45	♀	Hypothy	1.9	Hypothyroid	

RESULTS

Gastric secretion of acid

Basal secretion of acid was significantly lowered in ten patients with both hyper and hypothyroidism compared with controls (Figs 1 and 2). After histamine stimulation the acid output was within normal limits in two patients with hyperthyroidism (Figs 3 and 4). For all other patients the secretion of acid was significantly lowered.

Gastric secretion of intrinsic factor

Basal secretion of intrinsic factor was investigated in ten subjects with hyperthyroidism. In four patients the intrinsic factor secretion was lower than the lower range for hospital patients (2) (Fig 5).

In all patients with hypothyroidism the basal secretion of intrinsic factor was low in three subjects <100 units/h (Fig 6).

After histamine stimulation the secretion of acid

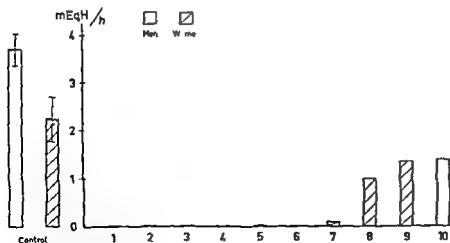


Fig 1 Basal secretion of acid in mEqH/h in ten patients with hyperthyroidism. Mean and S.E. for controls according to Dotevall (6).

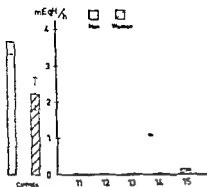


Fig 3 Basal secretion of acid in five patients with hypothyroidism. Mean and S.E. for controls.

trinsic factor was within normal limits in four patients with hyperthyroidism (Fig 7). In all other patients the secretion of intrinsic factor was low (Figs 7 and 8) in four patients with hyperthyroidism and three patients with hypothyroidism lower than the lower range for hospital patients (2).

DISCUSSION

Our findings of low acid output in patients with hypothyroidism and hyperthyroidism are in agreement with those of others. In an earlier publication (7) we found a good correlation between

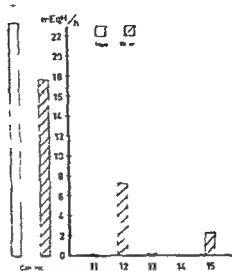


Fig 4 Gastric secretion of acid in mEqH/h after maximal histamine stimulation in five patients with hypothyroidism. Mean and S.E. for controls.

gastric acid secretion and gastric biopsy findings in patients with thyroid gland disease. Eight of 17 subjects studied with hyperthyroidism had atrophic gastritis and achlorhydria or highly depressed acid secretion after the augmented histamine test. In 15 patients with hypothyroidism

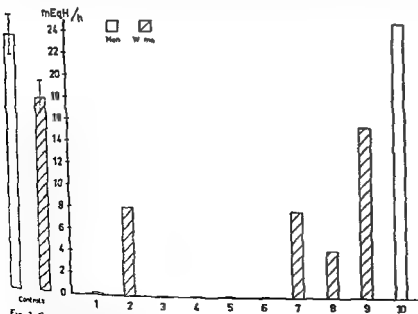


Fig 5 Gastric secretion of acid in mEqH/h after maximal histamine stimulation in ten patients with hyperthyroidism. Mean and S.E. for controls according to Dotevall (6)

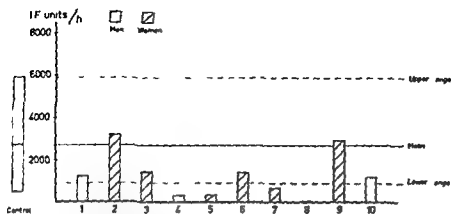


Fig 5 Basal secretion of intrinsic factor in units/h in ten patients with hyperthyroidism. Mean \pm range for controls according to Ardeman et al. (2)

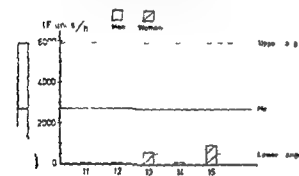


Fig 6 Basal secretion of intrinsic factor in units/h in five patients with hypothyroidism. Mean \pm range for controls

atrophic gastritis was seen in eight subjects who had achlorhydria or low acid output after the augmented histamine test

As far as hyperthyroidism is concerned a high

incidence of circulating antibodies to the gastric mucosa has been found (5). The report from Bock and Witts (3) of the connection between latent pernicious anemia, hypochlorhydria and achlorhydria in patients who had recovered from hyperthyroidism is of interest. In the present study three of the patients had had their hyperthyroidism some years before the gastric study and were euthyroid at the examination. Two of them (nos 3 and 4) had achlorhydria after the augmented histamine test and low values for intrinsic factor 1386 μ /h and 209 μ /h respectively. The Schilling test was however normal in both cases. All other patients had their hyperthyroidism diagnosed at the time of the study. Two patients were made euthyroid by thyroxin blocking drugs before the secretory test. They had a normal acid secretory response after histamine stimulation and the secretion of intrinsic

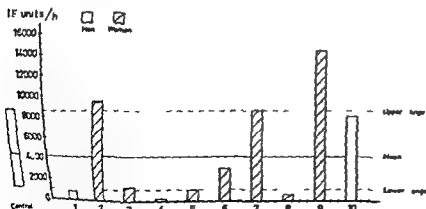


Fig 7 Gastric secretion of intrinsic factor in units/h after maximal histamine stimulation in ten patients with hyperthyroidism. Mean \pm range for controls according to Ardeman et al. (1)

ACKNOWLEDGEMENT

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CONCLUSION

The material of hyper and hypothyroidism presented here is small but the results confirm earlier findings of a low acid output in these conditions. The secretion of intrinsic factor correlates with the secretion of acid and is low in hypochlorhydria and achlorhydria. This is in agreement with the clinical association between thyroid gland disease and pernicious anaemia.

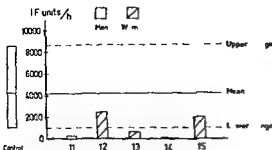


Fig 8 Gastric secretion of intrinsic factor in units/h after maximal histamine stimulation in five patients with hypothyroidism. Mean \pm range for controls

URINARY EXCRETION OF HYDROXOCOBALAMINE AND CYANOCOBALAMINE AFTER ORAL ADMINISTRATION OF LARGE DOSES

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Abstract Eight patients with pernicious anaemia in relapse have been treated with oral doses of 2000 μ g hydroxocobalamine twice daily. Remission was obtained in all patients within four to six weeks. Studies with labelled cobalamines showed that after large oral doses less OH B₁₂ than CN B₁₂ was excreted in the urine when equal doses were given to the same person. This difference was small but significant.

Hydroxocobalamine (OH B₁₂) is retained longer in the human body than cyanocobalamine (CN B₁₂) after parenteral administration (3, 5). Intestinal absorption in man of large doses of vitamin B₁₂ is not intrinsic factor dependent (2). At oral doses between 100 and 1000 μ g the mean absorption of OH B₁₂ and CN B₁₂ are not significantly different (6). Thus the greater body retention of OH B₁₂ would give a lower urinary excretion than that of CN B₁₂. In the present study the urinary excretion after large oral doses of the two forms of the vitamin has been compared.

METHODS

Routine haematological methods were used. 24-hour urines were collected in bags containing 10 mg of potassium cyanide; the volumes measured and frozen aliquots stored until assayed. The vitamin B₁₂ activity was estimated microbiologically with *Euglena gracilis* strains according to Hutner et al. (4). Radioactive oral doses were Co⁵⁷ labelled CN B₁₂ and Co⁵⁷ labelled OH B₁₂. Each tablet contained 1000 μ g of the vitamin with a radioactivity of 1 μ Ci. The urine was collected 0-24 h and 24-48 h after the oral dose. The radioactivity was measured with a scintillation counter in an arrangement for spectrometric analysis of Co⁵⁷ and Co⁶⁰ peaks.

RESULTS

The study was planned in four parts. In the first part eight patients with pernicious anaemia in

relapse were treated with oral doses of 2000 μ g of OH B₁₂ twice daily (Hepagon tablets 1 mg Astra). All patients were monitored by laboratory control. The reticulocyte response was measured; haemoglobin values together with serum B₁₂ estimations were determined every second week. Remission was obtained in all patients within four to six weeks (Fig. 1).

In the second part of the study oral doses of 2000 μ g of vitamin B₁₂ were given twice daily to male patients hospitalized because of uncomplicated myocardial infarction. The patients had no signs of haematological, hepatic or renal disease. The two forms of vitamin B₁₂ were given for one week each. Fig. 2 illustrates the variations in the 24-hour urinary excretions. No significant differences were found. It must be noted that the excretion was very low, less than 0.1% of a given daily dose.

In the third part of the study the urinary excretion of the two forms of vitamin B₁₂ were compared using equal oral doses of Co⁵⁷ labelled CN B₁₂ and Co⁵⁷ labelled OH B₁₂. The two forms were given simultaneously in doses from 1000 μ g to 4000 μ g each. A flush dose of 2000 μ g of non-radioactive CN B₁₂ or OH B₁₂ was given 2 and 24 h after the oral dose. Table 1 shows that less OH B₁₂ than CN B₁₂ was excreted. For ten patients receiving 4000 μ g daily of each form the mean 24-hour and 48-hour excretion was 4.7 μ g and 8.4 μ g respectively of OH B₁₂ and 7.2 μ g and 11.5 μ g of CN B₁₂. These differences in urinary excretions are highly significant (0.001 < p < 0.01).

Finally, daily oral doses of 1000 μ g Co⁶⁰ labelled CN B₁₂ or Co⁶⁰ labelled OH B₁₂ were administered on alternate days to two patients

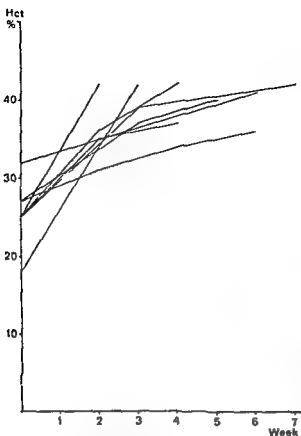


Fig 1 Increase in hematocrit concentration during oral treatment with 7000 µg of OHB twice daily

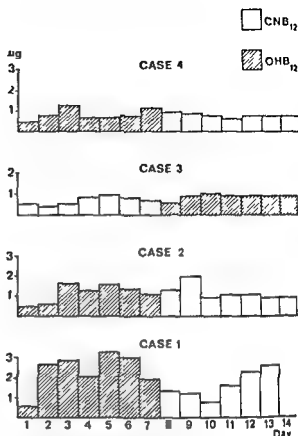


Fig 2 24 hour urinary excretion of vitamin B₁₂ following oral doses of 7000 µg twice daily OHB and CNB were given for one week each

Table 1 Urinary excretion of vitamin B₁₂ following identical oral doses of OH-Co⁵⁷-B₁₂ and CN-Co⁶⁰-B₁₂; dose given as 7000 µg of non radioactive OH B₁₂ in cases 1-8 and as CN B₁₂ in cases 9-13

Case no	Oral dose (μg)		0-24 and 0-48 hour urinary excretion (μg)			
			OH Co ⁵⁷ -B ₁₂		CN Co ⁶⁰ -B ₁₂	
	OH-Co ⁵⁷ B ₁₂	CN-Co ⁶⁰ -B ₁₂	0-24 hour	0-48 hour	0-24 hour	0-48 hour
1	1000	1000	3.8	4.9	4.2	5.5
2	1000	1000	0.6	0.9	1.4	2.1
3	2000	2000	3.1	4.5	5.2	7.3
4	4000	4000	3.1	5.4	5.5	8.1
5	4000	4000	2.5	4.9	5.8	10.0
6	4000	4000	2.6	5.2	4.0	6.5
7	4000	4000	6.0	10.0	10.3	15.6
8	4000	4000	4.0	10.4	5.9	12.8
9	4000	4000	4.3	5.5	7.5	9.3
10	4000	4000	2.8	6.2	3.0	6.5
11	4000	4000	9	17.9	9.3	12.8
12	4000	4000	8.5	10.2	14.3	16.8
13	4000	4000	4.0	13.0	6.0	16.4

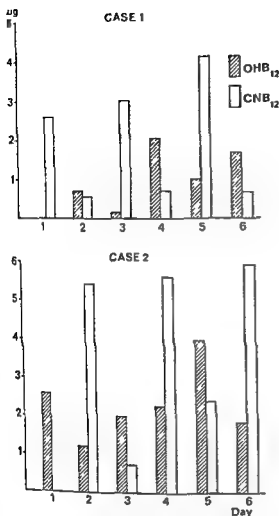


Fig 3 74 hour urinary excretion of vitamin B₁₂ following oral dose of 1000 μg OH-Co⁵⁷B₁₂ or CN Co⁵⁸B₁₂. CN B₁₂ given on days 1 3 and 5 in case 1 and on days 2 4 and 6 in case 2. OH B₁₂ given on the other days in each case. Daily flush dose containing 400 μg of each vitamin

for 6 days. A flush dose containing 500 μg of non-radioactive CN B₁₂ and 500 μg of OH B₁₂ was given 2 h after the oral dose. Fig 3 shows the urinary excretion values. The mean excretion of each vitamin for 5 days (3 days when tablets were given and the 2 days between these days) in case 1 was 1.1 μg of OH B₁₂ and 2.2 μg of CN B₁₂ and in case 2 2.3 μg of OH B₁₂ and 4.0 μg of CN B₁₂.

DISCUSSION

Using urinary excretion studies it has earlier been shown that after parenteral administration OH B₁₂

is retained longer in the human body than CN B₁₂ (5). This has been confirmed by Boddy et al (3) using whole body monitoring. They found a whole body retention of OH B₁₂ on the 3rd day of 42.1% after a single injection of 1000 μg. The retention of CN B₁₂ after the same dose was 13.7% by the 28th day the retention was 32.5% and 10.5% respectively. It is also shown that the absorption of large oral doses of the two forms of vitamin B₁₂ is not significantly different (6). Berlin et al (2) have shown that this absorption is approx 12% of the dose administered within a very wide dose range. Abe et al (1) gave oral physiological doses of the two forms to healthy persons. The faecal excretion rate did not show a significant difference. After a 1 mg flush dose given 2 h after the oral dose the 24 hour urinary excretion of OH B₁₂ was significantly lower than that of CN B₁₂ or 9.0 ± 1.9 and 26.7 ± 4.8 respectively of the given dose.

In the present study the urinary excretion after large oral doses of the two forms of vitamin B₁₂ was compared. Regardless of dose more CN B₁₂ than OH B₁₂ was excreted. 1000 μg is the recommended maintenance dose of CN B₁₂ in oral treatment of pernicious anaemia (2). This study has shown that OH B₁₂ is retained significantly better in the human body than CN B₁₂ even after large oral doses. However the difference is probably of little clinical importance and in the oral treatment of patients with pernicious anaemia the same doses of OH B₁₂ as of CN B₁₂ are recommended.

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EFFECT OF PARA AMINOSALICYLIC ACID ON SERUM LIPIDS

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Abstract The effect of PAS on serum lipids has been studied in a sample of 15 outpatients whose daily dietary regimes were not controlled. PAS was found to reduce serum cholesterol, phospholipid and total lipid values, a finding consistent with results reported earlier. Apparently owing to the freer dietary condition, the falls in lipid values in the present sample were somewhat lower than those reported earlier. Normal triglyceride values showed no significant fall but two patients with increased triglycerides showed considerable reductions. Further studies into the effect of PAS on triglycerides are obviously worthwhile.

A number of drugs have been used prophylactically against premature atherosclerosis but some of them have proved toxic to the human organism while others may have certain adverse side effects e.g. the thyroid preparations and female sex hormones. No completely satisfactory lipid reducing agent has been developed as yet.

The effect of PAS on serum lipids is less known. As early as 1955 Riska in his studies of Finnish tuberculosis sanatorium patients found that PAS definitely reduced serum cholesterol (10). This finding was confirmed by Tygstrup et al in 1961 from a sample of 24 non-tuberculous patients with hypercholesterolaemia (13) whose serum cholesterol fell by 30%. A detailed study was made by Kerstiell and Svanborg (8) in 1966. In addition to cholesterol they included in their study the other serum lipids viz phospholipids, triglycerides and total lipids. Their sample comprised seven patients and a remarkable fall was produced in all lipid fractions other than triglycerides which however were normal at the outset.

The purpose of the present study based on the above results was to make a supplementary examination of the action of PAS on serum cholesterol, phospholipids, triglycerides and total lipids in outpatients attending the Department of

Medicine University of Oulu. The sample consisted of selected patients whose serum cholesterol had already been determined for some reason and been discovered to be above the normal.

The sample comprised nine women and six men. Their cholesterol values ranged from 280 to 570 mg/100 ml. Their dietary regimes and customary ways of life were disregarded but they reported having been on low fat diets according to instructions received earlier.

The study was started by determining (1) cholesterol, (2) phospholipids, (3) triglycerides and (4) total lipids.

The patients then took PAS in the form of sodium salt for exactly one month, 12 g per day and a re-determination of the same lipids was carried out.

METHODS

Serum cholesterol was determined according to Abel et al (1), phospholipids according to van Handel and Zilverman (5), triglycerides according to Hawk et al (6) and total lipid according to Henry's method (7).

The normal values according to these methods are

cholesterol 170-270 mg/100 ml
phospholipids 1.5-3.5 mg/100 ml
triglycerides 60-100 mg/100 ml
total lipids 400-1000 mg/100 ml

RESULTS

The results are given in Figs 1 and 2. The lowered percentages were

cholesterol 17.5%
phospholipids 15.4%
triglycerides 20.0%
total lipids 18.8%

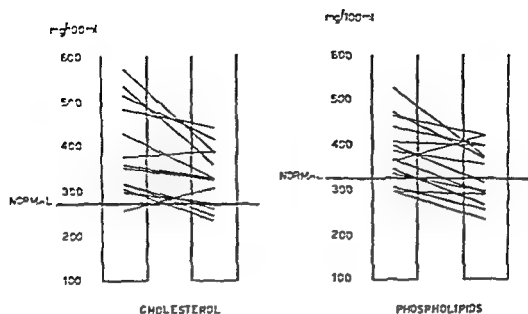


Fig. 1 The effect of PAS on serum of cholesterol and phospholipids.

The results agree with those reported from earlier studies even though the falls were less pronounced. The reason may be that the earlier studies had been carried out under controlled conditions in hospitals, keeping a rigorously fixed diet.

In two cases, with initial triglyceride values of 588 and 319 mg/100 ml the falls were 22.1 and 47% respectively (cases 3 and 7). It is therefore possible that PAS also reduces increased triglyceride levels a finding to encourage further studies of PAS on hypertriglyceridaemia. In triglyceride

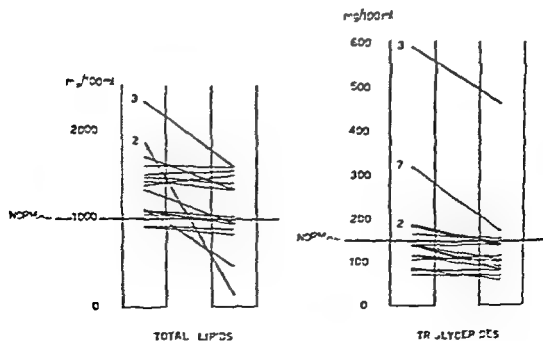


Fig. 2 The effect of PAS on serum level of total lipids and triglycerides.

Assumed second 1966

series the distribution of the falls was so wide that the result in this study is not statistically significant

DISCUSSION

The mechanism by which PAS affects serum lipids is not known with certainty. The assumption is that the steatorrhoea produced by PAS is the effective factor but Tygstrup et al (13) found the amount of steatorrhoea to be too low to account for such considerable reductions in serum lipids (13). Moreover in studies in vitro PAS did not reduce the cholesterol synthesis by the liver although this of course does not exclude a possibility of reduction in vivo. The effect of PAS on peripheral fat tissues is unknown. The salicylic acid chemically close to PAS reduces free fatty acids in the serum of both normal and diabetic subjects. This may result from reduced lipolysis (3). Whether or not PAS has a similar effect is unknown. Furthermore salicylic acid and its derivatives have been found to increase the turnover of thyroxine in the organism (14) whereas PAS has sometimes been found to produce hypothyreosis known to be associated with hyperlipaemia (2, 9).

It may be pointed out that in diabetes PAS has been found to reduce increased lipids (8, 24). It has even been reported in the light of four case reports that the wax like exudates in diabetic retinopathy decreased or disappeared in the course of PAS therapy (4).

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MECHANICAL HEMOLYSIS IN AORTIC VALVULAR DISEASE AND AORTIC BALL-VALVE PROSTHESIS

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Abstract Using serum haptoglobin concentration as an index, the frequency of hemolysis has been determined in 110 consecutive non-selected patients with aortic valvular lesions admitted to Medical Department B Rikshospitalet, during one year from February 1968. Marked lowering or absence of serum haptoglobin was seen in 11 of 65 patients with aortic valvular disease, hemolysis being about twice as frequent in aortic insufficiency as in aortic stenosis. Values indicating hemolysis were observed in 37 of 45 patients with a Starr Edwards aortic ball valve prosthesis. In most cases hemolysis was slight, no case with clinically important hemolytic anemia was seen. Marked lowering of the serum haptoglobin concentration seems to be the most sensitive qualitative indicator of hemolysis; the serum lactic dehydrogenase activity probably giving additional information about the rate of hemolysis.

Hemolysis due to mechanical trauma of red blood cells was previously seldom observed. After the introduction of open heart surgery however this problem has gained considerable interest (23). The early cases of hemolytic anemia following heart surgery were seen in patients operated for septum defects (12, 25, 29) and following plastic operations on the aortic and mitral valves (28, 30). Usually the hemolysis is moderate but fatal cases of hemolytic anemia are seen in patients with foreign material inserted permanently in the heart (17).

As early as 1956 Stohlman et al (27) showed that insertion of ball valve prosthesis in the aorta of dogs invariably caused hemolysis and in 1964 Dumeshek (5) observed hemolytic anemia in an unoperated patient with aortic valvular disease. During the last years numerous reports concerning hemolysis in aortic valvular disease and aortic valve prosthesis have been published (1, 2, 6, 10, 26, 32, 33) though most series are rather small and the data observed differ considerably (4, 7).

To get more precise information about the frequency and degree of the intravascular hemolysis in such patients we decided to study a larger group of non-selected consecutive patients suffering from different types of aortic valvular lesions and a reasonable number of patients after the insertion of a Starr Edwards aortic ball valve prosthesis.

MATERIAL AND METHODS

One hundred and ten patients admitted to Medical Department B during one year from February 1968 were examined, 65 cases with unoperated aortic valvular disease and 45 cases following insertion of a Starr Edwards aortic ball valve prosthesis. In 48 of the unoperated cases the clinical diagnosis was confirmed by left heart catheterization with measurement of the systolic gradient across the aortic valves; the degree of aortic regurgitation was determined by an independent observer performing cine angiography from the aortic root.

In the 65 unoperated patients aortic stenosis without insufficiency was found in 13 cases, and in 24 cases there was aortic insufficiency with a peak-systolic gradient less than 20 mm mercury across the aortic valves. Combined aortic stenosis and insufficiency was found in the remaining 8 cases; the relative importance of stenosis and insufficiency not being evaluated. The preoperative diagnosis in the 45 operated patients was aortic stenosis in 13 cases, aortic insufficiency in ten, and combined aortic stenosis and insufficiency in 22 cases. Minor mitral valvular lesion was observed in a few cases. Cases with mitral valvular lesion or other heart disease of significant importance were however not included in this study.

Hemoglobin concentration, blood cell counts, serum iron concentration and total iron binding capacity, serum haptoglobin concentration and the total serum lactic dehydrogenase activity were determined by routine laboratory methods. The haptoglobin concentration determined as the hemoglobin binding capacity of the serum normally ranges from 30 to 180 mg/100 ml. We used a value of 15 mg/100 ml or less as an indication of significant hemolysis. In normals the total serum lactic

Table I The distribution of the serum haptoglobin values in 65 cases with aortic valvular disease and 45 patients with a Starr Edwards aortic valve prosthesis

	No of cases	Serum haptoglobin concentration (mg 100 ml)			
		< 15	15-50	50-100	> 100
<i>Unoperated patients</i>					
Aortic stenosis	13	1	1	4	7
Aortic insufficiency	24	4	3	7	10
Aortic stenosis and insufficiency	28	6	6	9	7
<i>Operated patients</i>					
With a Starr Edwards aortic valve prosthesis	45	37	3	3	2

dehydrogenase activity is less than 200 units/liter. Reticulocyte counts, plasma hemoglobin and serum bilirubin concentrations were also determined. However, as these data did not give additional information about the hemolysis, the figures are not recorded here.

RESULTS

The haptoglobin values are shown in Table I. Low values indicating hemolysis were found in 14/20% of the unoperated cases, whereas the frequency was about 80% in the operated cases. The frequency of hemolysis in aortic insufficiency was about twice that seen in aortic stenosis. In patients with combined aortic stenosis and insufficiency, hemolysis was as frequent as in patients with pure aortic insufficiency. Hemolysis was most often seen in patients with marked

aortic insufficiency, whereas it was rarely observed in cases with a slight aortic regurgitation. However, the correlation between the degree of aortic insufficiency and the occurrence of hemolysis was not good, as several patients with marked aortic regurgitation showed no hemolysis. No relationship between hemolysis and the degree of aortic stenosis could be demonstrated.

In Table II the frequency of hemolysis in our patients is compared with data given by other authors. Of the series published hitherto, seven of the largest are included in the table. In three series the life span of ^{51}Cr -labeled red blood cells is determined, and in four series the serum haptoglobin values are used as an index of hemolysis.

Nearly half of the 45 operated patients were examined 2 to 4 weeks after the insertion of the aortic ball valve prosthesis. In 15 of these patients the examination was repeated 2 to 8 months later, and no significant change of the haptoglobin concentration was seen. Therefore we suggest that an observation period of 3 to 4 weeks following the operation usually is sufficient to evaluate the occurrence of hemolysis. Ball valve leakage due to suture insufficiency was seen in three cases, their haptoglobin values being 5, 10 and 15 mg/100 ml, respectively. Thus, in these patients hemolysis did not seem to be more severe than in the rest of the operated patients.

Fifteen patients were examined both pre- and postoperatively. Normal haptoglobin values were found in 13, and low values in two cases before the operation. Re-examination 3 to 4 weeks after the operation showed a normal haptoglobin value

Table II The frequency of hemolysis in the present series compared with figures given by other authors

Author	Method	Unoperated		Operated	
		No of patients	Hemolysis	No of patients	Hemolysis
Brodeur et al. (2)	$^{51}\text{Cr}^a$	21	7 (33%)	1*	11 (50%)
Westring (31)	$^{51}\text{Cr}^a$	1-	3 (5%)		
Yacoub & Keeling (33)	$^{51}\text{Cr}^a$			15	8 (53%)
Bell et al. (1)	Hp^b 0	11	2 (18%)	27	0 (74%)
Cuthbert (4)	Hp^b 13	11	2 (18%)	15	14 (93%)
Dupont & Wennebold (6)	Hp^b 33	7	5 (71%)		
Eysters et al. (7)	Hp^b 0	1*	2 (18%)	12	4 (33%)
Present series	Hp^b 15	59	11 (18%)	41	33 (81%)

* Half life of chromium-labelled red blood cells. Value indicating hemolysis arbitrary set to 0 days or less.

^b Serum haptoglobin concentration. Values used to indicate hemolysis by the different authors recorded in 15 x 15 x 4.

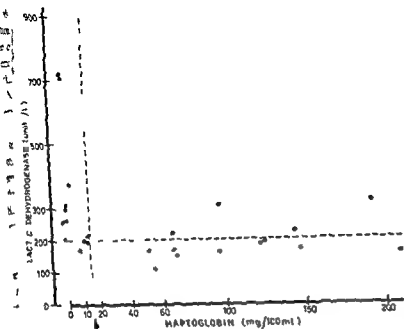


Fig 1 The relationship between the haptoglobin concentration and serum lactic dehydrogenase activity in 40 non-selected, consecutive cases.

in only one of these cases in the others there was a marked decrease of the serum haptoglobin indicating development of postoperative hemolysis.

The total lactic dehydrogenase activity of serum was determined in 50 cases. Fig 1 shows good agreement between these values and the serum haptoglobin concentrations. The lactic dehydrogenase activity was increased to more than 200 units/l in all but one of 24 cases with hemolysis as determined by a serum haptoglobin concentration less than 15 mg/100 ml. On the other hand in 26 patients with haptoglobin values exceeding 15 mg/100 ml the lactic dehydrogenase activity was less than 200 units/l in 20 cases. In three cases the increase in lactic dehydrogenase activity was slight and in two of three cases with marked elevation of the lactic dehydrogenase the increase might be explained by a possible myocardial infarction in one and a serious heart failure in the other.

Anemia was unusual even in cases with significant hemolysis as determined by serum haptoglobin and lactic dehydrogenase values. Hemoglobin values between 10.3 and 13.0 g/100 ml were seen in 11 of the 65 unoperated cases only in one of these could the anemia be explained by hemolysis. Irrespective of hemolysis iron deficiency was seen in several cases mainly in younger

women. In the operated patients anemia was more frequent. However because of the short observation period following operation in this group the relationship between anemia and hemolysis was not evaluated. No case of serious hemolytic anemia was observed in the present series.

DISCUSSION

There is a high proportion of combined aortic stenosis and insufficiency in the present series of unoperated patients, pure aortic stenosis being diagnosed in relatively few cases. The reason for this probably is that heart catheterization was performed in the majority of cases, thus giving a more precise diagnosis than the clinical findings alone.

Our data about the frequency of hemolysis in unoperated and operated patients with aortic valvular lesions are in agreement with those of most other authors. This may be surprising as different techniques and different normal values for serum haptoglobin have been used by some of the others. However as the serum haptoglobin usually falls to values close to zero even in slight hemolysis borderline values are recorded in relatively few cases.

In agreement with Brodner et al. (2) we found that hemolysis occurred twice as frequently in aortic insufficiency as in aortic stenosis. However these authors found a significantly higher frequency of hemolysis in the unoperated patients, whereas their figures in the operated cases were considerably lower. Our rather short postoperative observation period did not seem to influence the results. Therefore we have no other explanation of the different frequency found in the two series than the different techniques used. Several authors have observed increased hemolysis in cases with leakage around the prosthesis (1, 2, 18, 22, 23) even in patients with heterograft aortic valves leakage markedly results in hemolysis (21). In our material ball valve insufficiency occurred in only three patients in these the hemolysis was not more marked than in the rest of the operated cases.

The serum lactic dehydrogenase activity usually increased in hemolytic states this has also been observed in the hemolytic syndrome following aortic valve replacement (3, 19). Recently it has been pointed out (31) that the serum lactic dehydrogenase value seems to be a reliable quantitative index of hemolysis. We found elevated values in nearly all patients with intravascular

values. But as expected there were increased values also in a few cases without hemolysis. The enzyme in these cases most probably originated from the myocardium, kidneys and liver. However, our preliminary data (11) show a good correlation between the total serum lactate dehydrogenase value and the life span of red blood cells labeled with radioactive chromium. For clinical use we suggest that the L_{act} value should be used to determine whether hemolysis is present or not and that the serum lactate dehydrogenase activity is a simple and reliable parameter for evaluation of the degree of hemolysis. However, the L_{act} value shortly after blood transfusions and during venous infections such as bacterial endocarditis may be misleading. Furthermore, care should be taken in interpreting the L_{act} dehydrogenase value as myocardial, kidney and liver diseases have to be excluded before it can be used to evaluate the degree of hemolysis.

Other methods for determination of hemolysis, such as measurement of the carbonic hemoglobin in blood (9), serum bilirubin and plasma hemoglobin, likewise have failed in our hands. Others too

(20) have reported failure of the carboxyhemoglobin method in such patients.

Sears and Crohn (24) observed a consistent relationship between physical activity and the rate of hemolysis in two patients. Recently this has been confirmed by others (8, 31). We have studied the degree of hemolysis before and after a graded exercise test in several patients (16). Irrespective of the hemolytic activity in the resting state a slight increase in hemolysis was observed during work in patients with a ball valve prosthesis. Further study is necessary to correlate the increase of hemolysis with hemodynamic parameters.

The mechanism of hemolysis in these patients is still uncertain. In most cases a mechanical trauma undoubtedly is the cause but in some cases immunohemolytic anemia has been observed (13-15). As to the mechanical trauma, there is no agreement about the relative importance of direct trauma between the red cells and the valves and the injury of the red cells caused by the turbulent blood flow (14-19-22). The markedly different frequency of hemolysis in patients with aortic stenosis and aortic insufficiency could possibly be explained by the high pulse amplitude in the latter condition causing increased mechanical stress on the red cells not only in the heart and aorta but also in smaller arteries.

Our preliminary data (11) suggest a shortening of the red cell life span to between 50 and 75% of the normal a finding in agreement with other (1, 2). Thus the shortening of the red cell life span is so moderate that the bone marrow will compensate for it if iron or folic acid deficiency does not develop. The present material of 110 consecutive non-selected patients did not include any case with anemia of clinical importance. Therefore we conclude that, despite a high frequency of hemolysis severe anemia is seldom seen in patients with aortic valvular disease and in cases with Starr Edwards aortic ball valve prosthesis.

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HYPOGLUCOSURIA AND L FORMS OF ESCHERICHIA COLI
IN THE URINE

A Case Report

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Abstract. During screening for asymptomatic bacteriuria with the hypoglucosuria test, the urine from a seven-year old girl showed low glucose values, but negative conventional cultures. Heavy growth of L forms of *Escherichia coli* unaccompanied by growth of the classical bacterial form, was obtained on two occasions. The girl had no symptoms, no past history of urinary tract infection, and had never been treated with antibiotics. Shortly afterwards she presented symptoms suggestive of urinary tract infection, accompanied on this occasion by growth of classical *E. coli*. The finding of subnormal fasting urinary glucose levels in combination with negative conventional cultures may indicate a latent infection due to L forms.

L forms represent bacteria which lack a rigid cell wall. Because a rigid cell wall is lacking the L forms show pleomorphism and require a certain amount of osmotic protection in order to survive and multiply. On agar media L form colonies are characterized by a center portion becoming embedded in the agar. The center portion is surrounded by a peripheral growth on the agar surface which gives the familiar "fried egg" appearance. After primary induction from ordinary bacteria the L forms retain their ability to proliferate but they are usually unstable since they tend to revert to the bacterial form. The L forms may lose their tendency to revert after repeated subcultures (stabilized L forms).

Since the first report by Klineberger in 1935 (10) L forms have been isolated in various human infectious diseases such as bacterial endocarditis (6, 16, 21), meningitis (17, 22) and recurrent furunculosis (12). In 1965 Guttman et al. (13) isolated L forms from the urine of 11 of 57 selected patients with chronic urinary tract infec-

tion and pyelonephritis. Conner et al. (7) found L forms in 26 of 115 patients with recurrent urinary tract infections. However Guttman et al. (14) have suggested that urinary tract infections, associated with L forms, are probably uncommon. It has been proposed that the milieu in the renal medulla is osmotically favorable for the survival of L forms (4). Kalmanson and Guze (18) cultivated L forms from the renal biopsy material of seven patients. Five of these showed histological evidence of pyelonephritis. The patient described below was one of a series of 511 school girls between 7 and 18 who were studied for bacteriuria (26, 27). These studies and a previous investigation (25) showed that subnormal levels of urinary glucose, i.e. less than 1.5-2.0 mg per 100 ml, afford a reliable indication of significant bacteriuria. A test paper was developed which was sensitive enough to give a color reaction for glucose levels down to 1.5-2.0 mg per 100 ml of urine. The urine from eight of the 511 girls gave no color reaction with the test paper. The glucose concentration of these eight samples was less than 1.5 mg per 100 ml. Seven of the girls showed significant bacteriuria as defined by Kass (19). In contrast to these seven cases the urine from the patient reported on here yielded no growth of classical bacteria.

METHODS

The patient followed the instructions used in the survey (6) for the collection of urine. The patient had emptied her bladder on going to bed. Thereafter the patient had nothing to eat or drink until the sampling. The urine

ated as a mid-stream specimen from the first, fresh urine.

Urine glucose

The glucose concentration of urine was determined semiquantitatively by the test paper method (17) and quantitatively by the hexokinase-glucose-6-phosphate method (18). Test papers (Ungluc 2) from AB Kabi, Stockholm © Sweden.

Classical bacterial culture

Quantitative bacterial cultures were performed on blood agar plates by the calibrated loop technique.

OPMS

These were isolated on a solid medium heart infusion broth (Difco) 90 ml horse serum, 10 ml yeast extract, baker's yeast 25 g, 10 ml glucose, 20 g, 10 ml anducose 13 g. Antibiotics were not included. The pH was adjusted to 6.3 with hypochloric acid before adding 1 l of agar to the medium. The plates were incubated at 37°C in anaerobic jars in an atmosphere of 90% nitrogen and 10% carbon dioxide. Inoculated blood agar plates were incubated simultaneously in the same way to serve as controls. The plates were read under the microscope after 3 days of incubation and then every second day during a fortnight. Further observations were made under oil immersion, after agar blocks with growth of *L* forms had been prepared according to the wet-stained agar block technique (8). Subcultures were made by cutting out blocks of agar with growth of *L* forms. The blocks were placed face down and passed over the surface of fresh plates. For reversion of *L* forms to classical bacterial subcultures were made by the same technique.

Wet-stained agar plates

Micrological typing of isolated *E. coli* strains was done by Ida Olov M.D. at the International Escherichia Center Copenhagen, Denmark.

CASE REPORT AND RESULTS

The patient was a 7-year-old girl. Her past history was thoroughly investigated. She had previously been in good health, apart from infections of the upper respiratory tract. She had never been treated with antimicrobials, nor complained of symptoms suggesting urinary tract infection. Her family history was negative apart from the fact that a sister had Turner's syndrome.

The interval between the collection of the first two urine samples was 13 days. The urinary glucose concentration of the first sample was 0.6 mg per 100 ml and that of the second 0.5 mg per 100 ml. The pH was 6.3 and 5.6 respectively. As there was no growth of classical bacteria in the first sample and the urinary glucose concentration was subnormal, repeated cultures from the same sample were made on blood agar plates, and cultures for *L* forms were prepared. There was again no growth on the blood agar plates. On the *L* form medium, however, developing colonies, visible under the microscope, were found on the third day of incubation. The wet-stained agar block technique showed pleomorphic growth.

Within the first week of observation heavy growth of colonies was noted. They were granular in texture with a dense center embedded in the agar (fried egg morphology). Classical bacteria were not found on the *L* form medium. The control blood agar plates showed no growth. The colonies found could be propagated on *L* form medium by the agar block technique. When colonies were passed, in the same way over blood agar plates, the organisms reverted to classical *E. coli* after overnight incubation. Thus the organisms primarily isolated on the *L* form medium represented *L* forms and, after reversion, they did not grow as *L* forms when subcultured on *L* form medium. Cultures for mycoplasma, including T-422, were negative.

The results of cultivating the second sample collected from the patient were identical with those of the first sample. Thus, in two separate samples collected 11 days apart, the primary cultures from the urine showed heavy growth of *L* forms but no growth of classical bacteria. Also the *L* forms isolated from the second sample reverted to classical *E. coli*.

Two days after the collection of the second sample, the patient was requested to come to the Children's Hospital for a clinical examination. She had at that time no obvious tenderness over the right kidney. This tenderness had not been noted earlier by the patient. Otherwise the physical examination was normal. Blood urea nitrogen was 13 mg per 100 ml, WBC 3400 per cm³, ESR 3 mm per hour. The first morning urine showed a glucose concentration of 0.4 mg per 100 ml and an osmolality of 11 mOsm per kg. Urinary sediments were normal. Intravenous pyelography and voiding urethrocytography showed normal conditions. In contrast to the samples collected earlier the conventional cultures of the urine yielded, at this time, growth of classical *E. coli* with more than 100 000 organisms per ml. There was no growth of *L* forms. The *E. coli* strain from this third sample and the two strains which had reverted from *L* forms had all the somatic antigen O15. They were non-motile. They were also identical as fermentation of carbohydrates and showed the same pattern of sensitivity to antibiotics.

In vitro the organisms were sensitive to sulfonamides and sulfamoxol was administered. Ten days later exanthema developed and the treatment was changed to nitrofurantoin. Nitrofurantoin was given for 2 months. During the course of therapy and subsequently over another period of 18 months, repeated cultures yielded no growth of *L* forms or classical bacteria. The concentration of urinary glucose determined repeatedly during this period was always within the normal range and the test for glucose gave normal color reactions. The patient had no symptoms suggestive of urinary tract infection and repeated physical examinations were normal.

DISCUSSION

L forms may be produced when bacteria lose the ability to synthesize a normal cell wall. In vitro bacteria with a defective cell wall may be induced by antibiotics (11), lysozyme (32), microsomal fractions of leucocytes (3) and by antibodies

and complement (5) Also in vivo these and other host factors may account for the conversion of classical bacterial forms to L forms However in most instances where L forms have been isolated the subject had received antibiotics especially penicillins and often the parent bacterial form reappeared after the antimicrobial treatment was discontinued The case described had never been treated with antimicrobials before the L forms were isolated Among the conceivable factors accountable for the formation of the L forms lysozyme was searched for Repeated assays for lysozyme in the urine performed according to Parry et al (23) showed no measurable activity

Generally the defective cell wall makes the L forms dependent on a high environmental osmolality so as to escape lysis Braude et al (4) have stated that hypertonic urine may support survival of L forms and that the first morning urine is most suitable for isolation of L forms as the osmolality of morning urine is usually sufficiently high to prevent lysis That in our case isolation of L forms was successful may possibly be due to the specimens being collected from the first morning urine

The pathways for carbohydrate breakdown by L forms are less well defined Most information about the carbohydrate metabolism of L forms has been collected from studies of *Proteus* L forms According to Weibull and Beckman (33) the L forms have less oxidative activity than does the parent bacterial form On the other hand L forms seem to have a greater degree of glycolysis than the parent form In vitro glucose appears to satisfy the energy requirements of L forms (1) The subnormal levels of urinary glucose found in the case described indicate that L forms of *E. coli* localized within the urinary tract make use of the small amounts of urinary glucose normally present L forms of *E. coli* may survive and revert in the renal medulla of experimental animals (2) The hyperosmolality of the renal medulla was presumed to protect the L forms Experimentally Dienes and Weinberger (9) Silberstein (29) Freundt (10) and Gutman et al (15) were not able to demonstrate pathogenicity of L forms per se The pathogenicity returned when the L forms reverted to classical bacterial forms (10) In the present case there were no symptoms of urinary tract infection when the L forms were isolated However when the L forms had reverted tender

ness over the right kidney region was found Flank pain or tenderness is often known to be the presenting symptom of urinary tract infection in children 5-12 years of age (24-30) In the present case the flank tenderness in combination with bacteriuria suggests that the L forms primarily isolated gave rise to a dormant state of kidney infection which manifested when the L forms reverted to the parent bacterial form As Gutman et al (15) have pointed out however it is extremely difficult to relate L forms to human infections because the parent organisms are frequently isolated concomitantly with the L forms There fore in order to be able to relate L forms to disease in the host they must be isolated unaccompanied by classical bacteria preferably in the absence of antibiotic therapy thus indicating that L forms alone are capable of inducing disease These qualifications were satisfied by the patient described

The technique for isolation of L forms is both laborious and time consuming The detection of subnormal levels of urinary glucose in the presence of negative conventional cultures appears to be a useful means of selecting cases for L form studies

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DIAMETER OF THE RENAL ARTERY AND KIDNEY FUNCTION IN PATIENTS WITH PARENCHYMATOUS RENAL DISEASE RENAL ARTERY STENOSIS AND RENAL CARCINOMA

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Abstract In 79 patients (40 with parenchymatous renal disease 19 with unilateral stenosis of the main stem of the renal artery and ten with renal carcinoma) the diameters of the renal arteries (D) were measured by renal arteriography and the effective renal blood flow (ERBF) the insulin clearance (C_I) and the para-amino-hippuric acid clearance (C_{PAH}) were measured by the split function technique

In the patients with single renal arteries and parenchymatous renal disease positive curvilinear covariations between ERBF C_I C_{PAH} and D as well as D² were found. When the difference in function between the kidneys in the same individual (per cent of mean value) was correlated with the corresponding difference in D significant positive linear covariations between ERBF C_I C_{PAH} and D as well as D² were found in patients with single renal arteries and parenchymatous renal disease. There was no correlation between the functional parameters and D in the kidneys with renal artery stenosis or renal carcinoma.

It seems that D is so closely related to the blood flow through the renal artery when the variation of D is secondary to modifications in the parenchyma, that it can be used as approximate estimate of renal function.

In experimental hydronephrosis in the dog the diameter of the renal artery decreases passu with the area of the frontal projection the weight and the effective plasma flow of the kidney (6). In man the area of the frontal projection of the kidney has been reported to show a "logarithmic" covariation with the diameter of the renal artery (7). In patients with focal and diffuse kidney diseases the diameter of the renal arteries show a statistically significant covariation with the effective plasma flow of both kidneys (2).

With the intention of obtaining a more accurate description and a practical application of the relation between kidney function and the arterial

diameter the function of the individual kidneys has been compared with the arterial diameter in this study. The findings in disorders primarily affecting the renal parenchyma have been compared with the findings in renal artery stenosis which primarily affects the artery and in renal carcinoma, where the flow through the renal artery is shared between the cancer tissue and the remaining part of the kidney.

MATERIAL

The study comprised 40 patients with parenchymatous renal disease 19 patients with stenosis of the main stem of the renal artery and ten patients with renal carcinoma.

The relevant clinical data of the patients with parenchymatous disorders are given in Table 1. The diagnostic criteria are given elsewhere (3). As the renal artery is usually involved in general atheromatosis, the patients were grouped according to age and the presence of hypertension. Hypertension was defined as a blood pressure exceeding 160/100 mm Hg (cuff method). Eyeground changes exceeding group II of Keith and Wagener were not present in this patient group.

Eleven of the 19 patients with renal artery stenosis were men. The mean age in this group was 48.6 years (13 to 63 years). With the exception of one normal kidney all kidneys were supplied by single renal arteries. Eighteen patients were hypertensive eyeground changes corresponding to group III of Keith and Wagener were seen in two patients, and bilateral edema of the papilla was seen in one.

Four of the ten patients with renal carcinoma were men. The mean age in this group was 63.8 years (53 to 74 years). With the exception of one normal kidney all kidneys were supplied by single renal arteries. Three patients were hypertensive pathological eyeground findings were not present in this patient group. On the basis of the renal arteriogram the tumours were classified as having 1) an abundant vascular supply including the

Table I. Relevant clinical data in patients with parenchymatous diseases

The numbers of patients with unilateral renal disease are given in parentheses

	Normotensives (≤ 45 y)	Hypertensives (< 45 y)	Normotensives (≥ 45 y)	Hypertensives (≥ 45 y)	All groups
Sex Age (no. of patients/years)					
Male	8/25.5	5/32.2	2/56.5	3/50.3	18/34.9
Female	5/48.0	3/34.0	12/57.8	17/56.4	32/50.5
Patients with single artery bilaterally					
Diagnosis (no. of pairs)					
Pyelonephritis	4		8	7	19
Pyelonephritis and hydronephrosis	1			2	3
Hydronephrosis	1	2		1	4
Hypoplasia	2	1	1	1	5
Tuberculosis			1	1	2
Papillary necrosis		2			2
Hypertension				1	1
Patients with single artery unilaterally					
Pyelonephritis and hydronephrosis	1		2		3
Hydronephrosis	1			1	2
Tuberculosis				1	1
Hypertension		1	1		2
Patients with multiple arteries bilaterally					
Pyelonephritis and hydronephrosis			1		1
Tuberculosis	1				1
Total	12 (9)	8 (5)	14 (7)	15 (9)	50 (30)

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and 2) a moderate or sparse
erized by the presence of fewer
arteries in the tumour than in the
neighbouring tissue. All tumours were extirpated.
The tumours had as having a moderate or sparse
vascular supply either cystic (? patients) or grossly
necrotic.

With the exception of two patients (one with parenchymatous disease and one with renal artery stenosis) the serum concentrations of creatinine and urea were normal. The patients did not present symptoms or signs of electrolyte imbalance and were without signs of manifest heart failure.

METHODS

The procedure and methods of analysis and calculation used in measurements of C_{12} , C_{12H} and ERBF of the individual kidneys are described elsewhere (3).

The abdominal aortography was performed by percutaneous catheterization via the femoral artery and injection of the roentgen contrast medium (30 ml of 70 Isopaque) by the Gidlund pump. Six exposures were taken during the first 2½ sec after the injection, and an additional exposure 10 sec after the injection of the roentgen contrast medium.

The following diameters were measured on the arteriograms: 1) the diameter of the renal arteries (D_R) at the

point of first division; 2) the diameter of the abdominal aorta 1 cm central to the renal arteries; 3) the diameter of the abdominal aorta 1 cm peripheral to the renal arteries; and in patients with renal artery stenosis also 4) the minimal diameter of the stenosis. Correction for the average magnification (1.17 times) of the true dimensions was not made. All measurements were performed by the same investigator and by the use of the points of a pair of compasses mounted on a sliding gauge. A vernier scale on the sliding gauge permitted readings of 0.1 mm. On the basis of duplicate readings performed with an interval of several months the error of measurements ($d = d_n - d_n$, difference between duplicate readings, $n =$ number of duplicate readings) was found to be 0.43 mm for the diameter of the renal artery, 0.64 mm for the diameter of the abdominal aorta and 0.40 mm for the difference between the diameters of the renal arteries of the same individual.

Conventional methods (5) were used in the statistical treatment of the results.

RESULTS

Patients with parenchymatous renal disease and kidneys with a single renal artery

When C_{12} , C_{12H} and ERBF were plotted against D_{R1} or D_{R2} the distribution of the observations

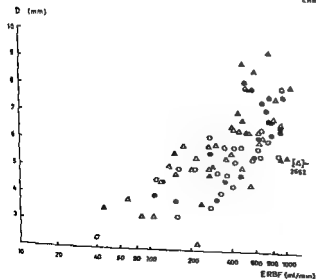
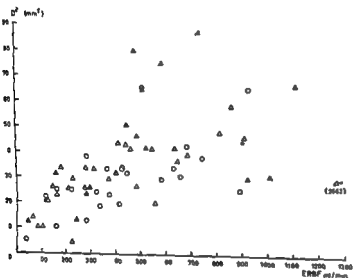
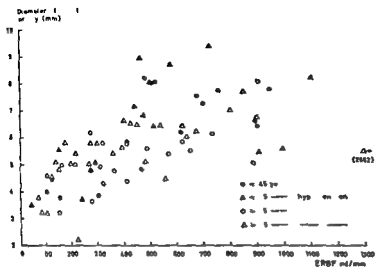


Fig 1 Relation between diameter of renal artery (D and D) and effective renal blood flow (ERBF linear and logarithmic coordinates) in patients with parenchymatous disorders

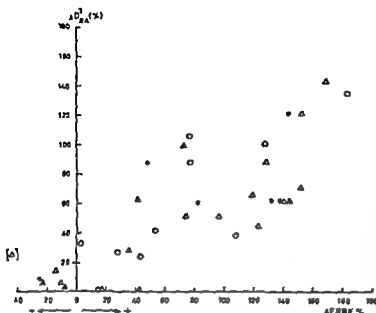


Fig 2 Relation between percentual difference of arterial diameter (ΔD_{RA}) and effective renal blood flow (ERBF) in the same individual in patients with parenchymatous disorders. Symbols as in Fig. 1

seemed to be curvilinear in the linear as well as in the semilogarithmic coordinate system (Fig. 1 Table II). A common feature was an increased scatter of the observations when ERBF exceeded 450 ml/min. C_{PAH} exceeded 250 ml/min and C_1 exceeded 35 ml/min.

Statistically significant correlations were found

between C_1 , C_{PAH} , ERBF and D_{RA} as well as D_{RA}^2 ($p < 0.05$, Table II). No effect of hypertension or age was observed.

When the difference in function between the kidneys of the same patient (per cent of mean value) was plotted against the corresponding difference between the arterial diameters (D_{RA} or D_{RA}^2),

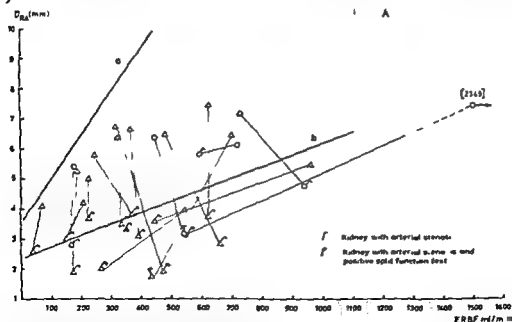
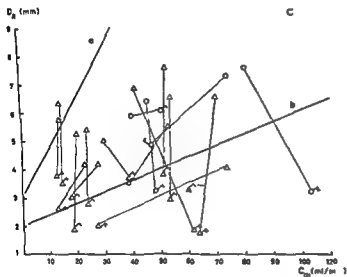
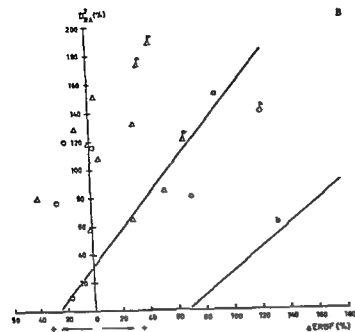


Fig 3 (A) Relation between diameter of renal artery (D_{RA}) and effective renal blood flow (ERBF) in patients with renal artery stenosis. (B) Relation between percentual difference of arterial diameter (ΔD_{RA}^2) and effective renal

blood flow (ERBF). (C) Relation between diameter of renal artery (D_{RA}) and inulin clearance (C_1). Symbol as in Fig. 1. a and b outline area of corresponding observations in patients with parenchymatous disorders.



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per cent of mean value) linear and highly significant ($p < 0.01$) correlations were observed (Fig 3 Table II)

The function was reduced below accepted limits of normality (4) when D_{ra} was less than 4 mm (12 kidneys $C_1 < 36$ ml/min $C_{PAH} < 190$ ml/min $ERBF < 320$ ml/min)

The kidneys with an arterial diameter of less than 3.5 mm were almost without glomerular filtration. The function was normal or only slightly

reduced when D_{ra} was greater than 6.5 mm (24 kidneys $C_1 > 30$ ml/min $C_{PAH} > 225$ ml/min $ERBF > 400$ ml/min). In the kidneys with 4 mm $< D < 6.5$ mm substantial reductions as well as great increases of the function were observed.

Patients with parenchymatous renal disease and multiple renal arteries in one or both kidneys

Putting D_{ra} (or D_{ra}) as equal to the sum of the individual diameters (or the sum of the squares

Table II Correlation between diameter of the renal artery and kidney function in patients with parenchymatous disorders

Δ = difference between the kidneys of the same individual (per cent of mean value)

y	x	Correlation coefficient	Regression equation
D_1	C_1	0.53	$y = 4.69 + 0.0389x$
D_{1a}^2	C_{1a}	0.37	$y = 25.4 + 0.26x$
D_{1b}	C_{PAR}	0.81	$y = 3.76 + 0.00795x$
D_2	C_{PAR}	0.72	$y = 18.27 + 0.0704x$
D_3	ERBF	0.53	$y = 4.27 + 0.00332x$
D_3^2	ERBF	0.32	$y = 26.47 + 0.070x$
ΔD	ΔC_{1a}	0.81	$y = 8.13 + 0.287x$
ΔD_{1a}^2	ΔC_1	0.78	$y = 19.00 + 0.480x$
ΔD_{1b}	ΔC_{PAR}	0.71	$y = 9.79 + 0.26x$
ΔD_3^2	ΔC_{PAR}	0.75	$y = 18.64 + 0.482x$

of these diameters) in kidneys with multiple renal arteries gave no correlation ($p > 0.10$) between the difference in diameter (D_{1a} or D_{1b} per cent of mean value) and the difference in ERBF or C_1 (per cent of mean value) between the kidneys of the same individual. The deviation from the observations in patients with single arteries on both sides was greatest in the patients in whom one kidney was supplied by two renal arteries of about equal diameter.

Patients with renal artery stenosis

When C_1 , C_{PAR} and ERBF were plotted against D_{1a} or D_{1b} the observations in kidneys without artery stenosis corresponded with the findings in patients with parenchymatous disorders (Fig. 3). The observations in the kidneys with stenosed

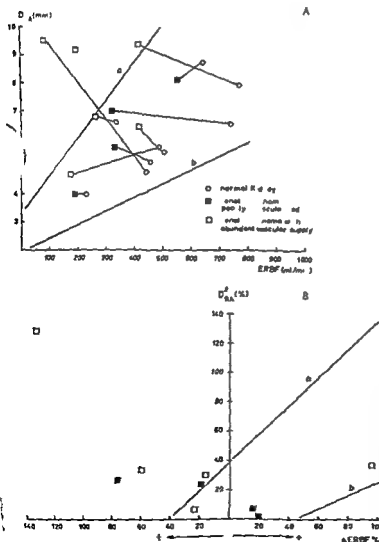


Fig. 4 (A) Relation between diameter of renal artery (D) and effective renal blood flow (ERBF) in patients with renal artery stenosis. (B) Relation between percentage difference of arterial diameter (D) and effective renal blood flow (ERBF) in the same individual. 'a' and 'b' outline area of corresponding observations in patients with parenchymatous disorders.

arteries differed from the latter showing no significant correlation between function and arterial diameter (D_{ra} or D_{ra}). At equal D_{ra} the blood flow in a stenosed artery was greater than in an artery without stenosis. There seemed to be no difference between the observations in kidneys involved in a positive split function test (4 kidneys with stenosis) and the other kidneys with stenosis of the renal artery.

Patients with renal carcinoma

When C_{1-5} , C_{RAH} and ERBF were plotted against D_{ra} or D_{ra} the observations in the normal kidneys were in correspondence with the findings in patients with parenchymatous disorders (Fig. 4). The observations in four out of six kidneys with carcinoma with an abundant blood supply fell outside the range of observations in the group with parenchymatous disorders. The ERBF in kidneys with carcinoma tended to be less than in a kidney without carcinoma at equal D_{ra} .

Patients with parenchymatous disorders. Difference between the aortic diameters 1 cm central and 1 cm peripheral to the origin of the renal arteries and ERBF

No significant correlation was found between the effective blood flow leaving the abdominal aorta through the renal arteries and the difference in diameter of the abdominal aorta central and peripheral to the origin of the renal arteries.

DISCUSSION

The present investigation confirms earlier assumptions of a positive correlation between the diameter of the renal artery and the blood flow through the artery. It would seem that this correlation is not absolute but depends on the modification in the renal artery being secondary to modifications in the parenchyma. The lack of correlation between function and diameter in the stenosis group is probably due to most of the stenoses not being particularly pronounced; it is well known that slight stenoses have little haemodynamic effect. On the other hand the lack of correlation between the diameter of the renal artery and ERBF, C_{RAH} and C_1 in the diseased kidney of cancer patients shows that the diameter of the renal artery is not a function of the amount of functioning renal tissue but of the blood flow

through the artery in the kidneys in which the vascularization of the tumour was abundant. ERBF was surely much less than the blood flow through the kidney.

As the functional parameters were better correlated to D_{ra} than to D_{ra} , the cross section of the artery does not appear to be the dimension that gives the best expression of changes in the blood flow through the kidney. The findings in kidneys with multiple arteries point in the same direction.

It seems reasonable to interpret the relation between the diameter and blood flow as an indication that the diameter of the renal artery depends on the amount of blood usually flowing through it. The correlation found between the diameter and the blood flow will thus be affected by the investigation situation to the extent this causes a deviation from the usual blood flow of the kidneys. Taken in conjunction with the relatively great measuring error in the diameter measurements, this may explain the scatter of the observations, especially at the highest ERBF values. Unusual RBF in some patients during the selective function test may also explain why the correlation between the diameter of the renal artery and ERBF was curvilinear when the data for all kidneys were pooled (Fig. 1) and linear when based upon the differences between the diameters and the ERBF of the kidneys in the same individual (Fig. 2). If ERBF deviated from the usual flow in the same way in both kidneys in these patients, the change of the percentual difference in ERBF would be relatively slight even on a considerable change in blood flow. However, unusual flow rates in some patients provided that the corresponding variation of D_{ra} was small might transform a linear correlation between ERBF and D_{ra} into a curvilinear one when the absolute flow rates are plotted against absolute values for D_{ra} .

It seems that D_{ra} is so closely correlated to the insulin clearance and the blood flow through the kidneys in patients with parenchymatous disorders that D_{ra} in kidneys with single arteries can be used as an approximate estimation of the renal function in these patients. A common problem in urology is the question whether a kidney should be resected or removed. As a guiding rule kidneys with D_{ra} of less than 3 mm can be removed with a loss of only 10 ml of glomerular filtrate or less; kidneys with D_{ra} of 6 mm or more can tolerate

an ordinary kidney, resection of $\frac{1}{3}$ or $\frac{1}{4}$ of the kidney mass

The investigation cannot answer the question of why the diameter of the renal artery is reduced even in minor reductions of blood flow. In view of the fact that the pathological modifications in several of the patients were unilateral it would seem reasonable to seek local causes. In this connection it is of interest that Hilton (1) has demonstrated local modifications not of nervous origin in the volume of the femoral artery of the cat on modification of the peripheral blood flow in the extremity concerned.

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SERUM B_{12} AND SERUM IRON AFTER GASTRIC SURGERY

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Abstract Immediately after Billroth II one third of patients have lost intrinsic factor most of them permanently. As time goes on there is a steadily rising incidence 45% lacking intrinsic factor after 18 months. Impaired absorption of labeled vitamin B_{12} is more frequent after surgery for stomach cancer than in patients with gastric or duodenal ulcer. Loss of intrinsic factor is less pronounced in duodenal than in gastric ulcer.

The pattern of serum iron levels gradually becomes similar to that of pernicious anemia after surgery. All patients operated on should be kept under medical observation, so that vitamin B_{12} and iron can be supplemented at the most favorable time.

The benefits of gastric surgery are many including elimination of life threatening complications such as perforation hemorrhage and stenosis. In recent years however there has been a wider consideration of ill effects such as dumping bilious vomiting and hypoglycemia on the one hand and metabolic disorders such as vitamin B_{12} hypovitaminosis including neurological and mental disturbances osteoporosis steatorrhea and hypoproteinemia.

According to Deganello (2) Moynihan (9) and Hartmann (5) the complete picture of pernicious anemia after total gastric resection will appear after 2-3 years or even more. As a first sign according to Pitney and Beard (10) the serum B_{12} will diminish then follows macrocytosis and finally megaloblastic anemia.

Earlier Lous and Schwartz (7) from Denmark in a study from 1959 of fifty-one patients with partial gastrectomy for duodenal ulcer found reduced absorption of vitamin B_{12} in seven. In 15 out of 45 patients operated on for gastric ulcer and 13 out of 17 patients operated on for cancer of the stomach the labeled vitamin B_{12} absorption test was impaired. The average observation period was six years.

In 1965 Deller (3) published a study comprising 365 patients. The selection was made at random from hospital records and reexamination. The male/female ratio was 23/1. duodenal/gastric ratio 14/1 and mean interval after surgery seven years. Sixty-one had radioactive vitamin B_{12} absorption tests and fifty seven bone marrow biopsies. The marrow was megaloblastic in 2% and transitional megaloblastic in 5%. A low serum vitamin B_{12} level was found in 13%. The absorption of labeled vitamin B_{12} was impaired in more than one third. The serum iron level was reduced in one third of subjects and anemia defined as a level less than 136 g% in men and 116 g% in women was detected in one-quarter. Mainly then the anemia was due to iron deficiency although a deficiency of vitamin B_{12} was also apparent in some cases.

It might therefore be of some consequence if one could at an early stage foresee which patients are likely to develop vitamin B_{12} hypovitaminosis after gastric surgery.

MATERIAL

To fulfil this object, we have collected sixty-three patients (69 men and 14 women) operated on with Billroth II. Twenty-five patients had gastric ulcer (19 men and 6 women) including one woman with protein losing gastritis. Twenty-seven patients had duodenal ulcer (21 men and 6 women). Eleven patients had carcinoma of the stomach (9 men and 2 women).

METHODS

All patients underwent a series of blood examinations. Schilling's test serum iron and vitamin B assay immediately after gastric surgery and every 6 months afterwards the last of them performed after two years.

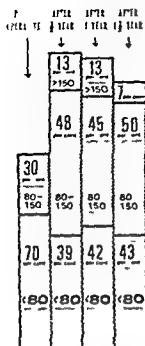


Fig. 1 Percentage of serum iron values $>150 \mu\text{g}$ 80 to 150 and less than 80 $\mu\text{g}\%$ post-operatively at 1 and 5 years after Billroth II

The labeled B_{12} absorption test was carried out according to Schilling's (13) urinary excretion method after oral administration of $0.4 \mu\text{g}$ of ^{57}Co -labeled vitamin B_{12} . One mg of cold vitamin B_{12} was injected 1 ml i.v. and the radioactivity of the 24 h urine specimen was measured with a well type scintillation counter Ekco 564 A.

RESULTS

Twenty-one patients (18 men and 3 women) out of 63 (33%) had a Schilling's test of 10% or lower immediately after gastric surgery. Among those with impaired absorption of radioactive vitamin B_{12} , 10 of 26 had been operated on for gastric ulcer, 1 for protein losing gastritis, 5 of 26 for duodenal ulcer, and 6 of 10 for cancer of the stomach. In other words, the risk of loss of intrinsic factor is far greater after partial gastrectomy for cancer or ulcer of the stomach than for duodenal ulcer. These findings agree with the observation of Lous and Schwartz (7) albeit they maintain that the loss of intrinsic factor is a fairly established fact immediately after surgery in one third of patients. Three of twenty-one had already at this stage a low serum vitamin B_{12} value less than 100 pg/ml . Among the remainder only two had so low a value.

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After 6 months twenty-six (21 men and 5 women) of 61 (43%) had a Schilling's test of 10% or lower.

Thirteen patients with a normal Schilling's test immediately after gastric surgery presented a pathologic test whereas eight patients with a pathologic test (of 10, 10, 8, 7, 5, 4, 3 and 1%) had a normal test after 6 months. It would seem reasonable to suggest that the high number of pathologic tests immediately after gastric surgery could be explained by a pathologic test already before the operation. This suggestion must be refuted as we know that patients with duodenal ulcer have a higher and patients with gastric ulcer of the body of the stomach a lower secretion of intrinsic factor than normal, but usually they do not lack intrinsic factor according to Doscherholmen (4). Moreover, as many as eight patients changed from a pathologic to a normal test later on, and it therefore seems likely to suppose that such patients due to the trauma inflicted by surgery lost their ability to produce intrinsic factor for a limited time.

This should be taken with some reservation, however. As a matter of fact five patients had a low serum B_{12} value immediately after surgery. Two of them had a pathologic Schilling's test both postoperatively and during the whole period of observation. The same holds true for another case. Being a case of cancer of the stomach it is easier to understand that there may be a low of intrinsic factor at the time of operation. The remaining two patients had a normal Schilling's test immediately after gastric surgery and later on.

There are ten cases of malignancy in this study. Eight of ten had a pathologic Schilling's test, two had not. A pathologic Schilling's test therefore seems to occur more often in patients with stomach cancer than in the rest of the material studied here. 18 of 52 with stomach or duodenal ulcer.

Evidently there is a relatively high chance for patients with stomach cancer to develop a vitamin B_{12} hypovitaminosis when operated on, whereas this risk seems to be much less in patients with gastric or duodenal ulcer. Ten of twenty-six operated on for gastric ulcer, five of twenty-six for duodenal ulcer, six of ten for cancer of the stomach had impaired absorption of labeled vitamin B_{12} . The loss of intrinsic factor at this stage

is remarkable for the group of cancer patients and somewhat more pronounced for gastric than for duodenal ulcer patients.

After one year 31 of 51 (21 men and 10 women) 61% had a pathologic Schilling's test. Ten of them were new cases who had presented a normal test at the previous two controls. The proportion of impaired absorption at this stage was fourteen of twenty-one for gastric nine of twenty-two for duodenal ulcer and eight of eight for stomach cancer. There is a distinctly higher number with intrinsic factor loss in the gastric than in the duodenal ulcer group. In the group of stomach cancer the whole group has impaired absorption of labeled vitamin B_{12} .

After 18 months only forty patients have hitherto been reexamined: eighteen of them 45% (11 men and 7 women) with a pathologic Schilling's test. Four of them were cases with a previous history of a normal test. Three of them remained pathologic 6 months later whereas the fourth has not yet been reexamined. At this stage (18 months) there is a still much higher loss of intrinsic factor in the gastric ulcer than of nine tests than in the duodenal ulcer group: three of fifteen. Five of the six patients with partial gastrectomy for cancer had a pathologic Schilling's test.

Iron deficiency

In the present study a normal serum iron level (80 to 150 $\mu\text{g}\%$) was found in 30% and subnormal level (less than 80 $\mu\text{g}\%$) in 70% postoperatively. The incidence of subnormal serum iron level therefore is much higher than the incidence of pathologic Schilling's test and of subnormal B_{12} vitamin level in the blood (Fig. 1).

After 6 months a normal serum iron level was found in 48%, a subnormal level in 39%, and a high level (more than 150 $\mu\text{g}\%$) in 13%. After one year the corresponding levels are 45%, 42% and 13% and after 18 months 50%, 43% and 7%. After two years we have hitherto got 24 controls only too few to permit definite conclusions. There seems however to be a tendency to fewer high levels than before whereas the number of normal and subnormal levels is about the same as after 6 months and 1 year.

On the whole we may conclude that there is a distinct tendency to higher normal and high serum iron levels and a lower number of sub-

normal values after 1, 1 and 1 1/2 year and probably a tendency to lower high levels later on.

It is well known that the serum iron level is definitely increased in untreated cases of pernicious anemia. After treatment there is a sharp drop of iron level to normal or even very low levels during the first 24 to 48 hours (6, 8, 14).

It seems reasonable to suppose that the behavior of the serum iron after gastric surgery reflects this specific tendency to establish the picture usually found in pernicious anemia: a tendency which if registered in patients after Billroth II is a warning to institute proper treatment. This tendency in our study is evident in spite of a few cases in which treatment with vitamin B_{12} was started as a consequence of low B_{12} levels registered initially or later during this study.

DISCUSSION

In conclusion we may state as a most important finding that immediately after Billroth II not less than one third of the patients have a Schilling's test of 10% or lower and in thirteen of sixty-three the test remains low later on. On the other hand in eight cases a pathologic Schilling's test immediately after gastric surgery becomes normal later on.

After 1, 1 and 1 1/2 year there is a higher incidence of pathologic Schilling's test from 33% immediately after gastric surgery to 43% after 1 1/2 year, 61% after 1 year and 45% after 1 1/2 year. In other words after 1 1/2 year about 45% of patients in our study lack intrinsic factor and therefore are inclined to develop pernicious anemia. This tendency seems to continue for two years but due to the limited number of observations (22 only) we refrain from further evaluation.

It might be suggested that a certain number of patients operated upon may have a pathologic Schilling's test and low serum vitamin B_{12} levels before the operation. Except for a few cases this seems highly improbable. In some instances operational trauma may favor a transitional loss of intrinsic factor.

Impaired absorption of labeled vitamin B_{12} is more frequent in patients with stomach cancer than in patients with gastric or duodenal ulcer. After 1 and 1 1/2 year practically all cases of stomach cancer still alive have lost their intrinsic factor. As time goes on the

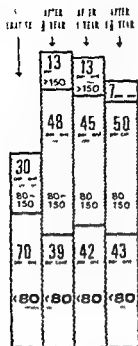


Fig. 1 Percentage of serum iron values $>150 \mu\text{g}$ 80 to 150 and less than $80 \mu\text{g}$ post-operatively 0, 1 and 2 years after Billroth II

The labeled B₁₂ absorption test was carried out according to Schilling's (13) urinary excretion method after oral administration of $0.5 \mu\text{g}$ of ^{57}Co labeled vitamin B₁₂. One mg of old vitamin B₁₂ was injected 1 h later and the radioactivity of the 4 h urine specimen measured with a well type scintillation counter Ekco

RESULTS

Twenty-one patients (18 men and 3 women) out of 63 (33) had a Schilling's test of 10% or lower immediately after gastric surgery. Among those with impaired absorption of radioactive vitamin B₁₂, 10 of 26 had been operated on for gastric ulcer, 1 for protein losing gastritis, 5 of 26 for duodenal ulcer, and 6 of 10 for cancer of the stomach. In other words the risk of loss of intrinsic factor is far greater after partial gastrectomy for cancer or ulcer of the stomach than for duodenal ulcer. These findings agree with the observation of Lous and Schwartz (7) albeit they maintain that the loss of intrinsic factor is a fairly established fact immediately after surgery in one third of patients. Three of twenty-one had already at this stage a low serum vitamin B₁₂ value less than 100 pg/ml. Among the remainder only two had so low a value.

After 6 months twenty-six (21 men and 5 women) of 61 (43%) had a Schilling's test of 10% or lower.

Thirteen patients with a normal Schilling's test immediately after gastric surgery presented a pathologic test whereas eight patients with a pathologic test (of 10, 10, 8, 7, 6, 4, 3 and 1%) had a normal test after 6 months. It would seem reasonable to suggest that the high number of pathologic tests immediately after gastric surgery could be explained by a pathologic test already before the operation. This suggestion must be refuted as we know that patients with duodenal ulcer have a higher and patients with gastric ulcer of the body of the stomach a lower secretion of intrinsic factor than normal, but usually they do not lack intrinsic factor according to Doscherholmen (4). Moreover as many as eight patients changed from a pathologic to a normal test later on and it therefore seems likely to suppose that such patients due to the trauma inflicted by surgery lost their ability to produce intrinsic factor for a limited time.

This should be taken with some reservation, however. As a matter of fact five patients had a low serum B₁₂ value immediately after surgery. Two of them had a pathologic Schilling's test both postoperatively and during the whole period of observation. The same holds true for another case. Being a case of cancer of the stomach it is easier to understand that there may be a loss of intrinsic factor at the time of operation. The remaining two patients had a normal Schilling's test immediately after gastric surgery and later on.

There are ten cases of malignancy in this study. Eight of ten had a pathologic Schilling's test, two had not. A pathologic Schilling's test therefore seems to occur more often in patients with stomach cancer than in the rest of the material studied here: 18 of 52 with stomach or duodenal ulcer.

Evidently there is a relatively high chance for patients with stomach cancer to develop a vitamin B₁₂ hypovitaminosis when operated on, whereas this risk seems to be much less in patients with gastric or duodenal ulcer. Ten of twenty-six operated on for gastric five of twenty-six for duodenal ulcer six of ten for cancer of the stomach had impaired absorption of labeled vitamin B₁₂. The loss of intrinsic factor at this stage

PLASMA CELL NEPHROPATHY WITH RENAL TUBULAR ACIDOSIS

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Abstract A case of renal tubular acidosis in a 34 year-old man with electrolytic disturbances, nephrocalcinosis and osteomalacia, and a long history of hypergammaglobulinemia with a positive Waaler Rose test is described. Surgical biopsy showed plasma cell infiltrates in the kidney. It is suggested that renal tubular acidosis might sometimes be a manifestation of an autoimmune disease, plasma cell nephropathy.

The clinical picture of renal tubular acidosis was first described in the mid 1930s (3-9) since when the condition has also been seen in adults. In most of the cases the gamma globulin concentration was also raised (1-16). Co-existing purpura, hyperglobulinemia of the type described by Waldenström in 1944 (14) has also been reported (4-7). After tubular defects had been demonstrated in Sjögren's syndrome (8-11) and in myelomatosis (5-10) the capacity of the kidneys to acidify the urine in the presence of hyperglobulinemia was studied systematically by Curtis Morris Jr and Fudenberg (6). In several cases of hypergammaglobulinemia of varying etiology they found that the ability of the kidney to acidify the urine was reduced. The mechanism of this disturbance was obscure.

The histological changes in a case seen at our department might perhaps provide a clue to the relation between hypergammaglobulinemia and renal tubular acidosis at least in polyclonal gammopathy. This case is reported below.

CASE REPORT

The patient was a man born in 1929.

His father born in 1898 and his mother born in 1905 enjoyed good health, and at examination in 1968 the concentrations of the serum gamma globulins and electrolytes were normal.

During adolescence the patient appeared to be slightly mentally deficient. He felt well until 1955 when he developed diffuse abdominal pain for which he sought advice at the department. The only noteworthy findings were hypergammaglobulinemia (19 g/100 ml) and a positive Waaler Rose test. In 1964 he had an acute attack of left-sided abdominal pain. Urography then showed nephrocalcinosis. The hypergammaglobulinemia was unchanged. Serum creatinine 1.3 mg/100 ml. Serum bicarbonate 18 mEq/L. Serum potassium 3.1 mEq/L. Specific gravity of urine 1.007 in concentrated samples. Alkaline phosphatase 28 units. Repeated attacks of renal colic until 1967. In Feb 1967 vomiting, muscle weakness and abdominal pain supervened, and the patient deteriorated rapidly. On admission to the Medical Department the patient was very ill with muscular adynamia and paralytic ileus. BP 125/80. Serum electrolytes (mEq/l): sodium 138, potassium 1.8, chloride 119, bicarbonate 10, calcium 4.0. Phosphorus 0.7 mg/100 ml. pH of urine 7.0. pH of blood 7.30. No demonstrable aldosterone in urine. Alkaline phosphatase 15 units. Serum creatinine 1.1 mg/100 ml, Hb 9.6/100 ml. ECG showed grave electrolytic disturbances. After correction of these abnormalities the patient improved promptly and gained 15 kg within a few months. His weight was then normal. Supplementary examinations were carried out during the period of clinical improvement.

Kidneys

Polyuria, about 3 l a day. Specific gravity after restriction of fluid 1.009. pH of urine repeatedly about 7.0. No fall of pH after administration of ammonium chloride in a dose of 0.1 g/kg body weight. As a rule the urine contained no protein or bacteria. A stone was passed on one occasion. Analysis of stone: calcium, phosphate and oxalate. No glycosuria. Examination of amino acids revealed nothing remarkable.

Aldosterone in urine: 0-52-148 µg/day. Creatinine clearance 70 ml/min. Phosphate reabsorption 75%. Roentgen examination of the kidneys showed as before nephrocalcinosis.

Skeleton

Biopsy specimen of the iliac crest after administration of tetracycline revealed severe active renal os-

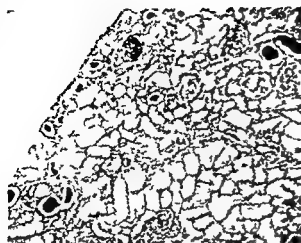


Fig. 1 Dense inflammatory cellular infiltrates in the cortex. Some tubules are atrophic and some contain colloid material. A few glomeruli are atrophic. H.E., $\times 53$.

... the fibro-osteoclasts. Deposition of tetra-
... extremely scanty in relation to the widespread
... (Prof. E. Uehlinger).

... the calcium metabolism with ^{45}Sr showed
... all large increased secretion and an
... of exchangeable calcium. Roentgen exami-
... of osteoporosis with certainty.

... renal with Hb of about 11 g/100 ml
... normal B₁₂ marrow of normal com-
... of the iron metabolism with ^{59}Fe
... the remarkable total iron metabolism in the
... kg body weight daily incorporation 100%
... Vitamin B₁₂ and acid in the blood.

Free HCl g/100 ml in feces 3 g/day. Xylose
and lactose tolerance test. Schilling test 17% excretion
in 4 h after 100 μCi in 1 ml urine.

Serological analysis

Gamma globulins about 1000 ml in several specimens
(increased polyclonal). Waaler Rose 1:8. FFA reaction 1260. Immune fluorescence examination positive for
cell nuclei and smooth muscle and negative for thyroid,
parietal cells and kidney.

Renal biopsy

Surgical biopsy with removal of two pieces from the
lower pole of the right kidney was done by Dr Land-
stedt, Urological Dept., Laartorget, Lund. The specimens
were examined histologically by Prof. F. Linell.

The specimen consisted of cortex only. The most
salient findings were numerous interstitial cell infiltrates
built up mainly of plasma cells with a moderate admixture
of lymphocytes. Many of the infiltrates were confined to
the superficial part of the cortex but some extended

cord like into the deeper layers of the tissue. Most of
the infiltrates were situated around glomeruli. There
were glomerular changes in the infiltrated areas. The
specimen showed all stages of periglomerular fibrosis,
which in some areas encroached upon the glomeruli, some
of which had undergone hyalinisation. Adjacent to the
infiltrated areas were atrophic tubules, some of which
contained colloid like masses. In some areas there were
signs of urinary reflux with concentrated urine in partly
widened ducts. Around these areas was a moderate pro-
liferation of fibroblasts and incipient formation of giant
cells in the tubular epithelium. Interstitially there were a
few calcium granules but otherwise no signs of nephro-
calcinosis. Between the abnormal areas were large regions
of well preserved cortical parenchyma with essentially
normal glomeruli and well preserved tubules. No obvious
vacuolisation of the cells of the tubuli. No angitis. (Figs.
1 and 2).

Course

During follow up (about two years) the patient's general
condition and working capacity were good. He occasion-
ally had brief spells of hematuria in connection with the
passage of stones. Apart from mild hyperchloremia the
acid base balance was controlled by administration of
alkalising salts (potassium sodium lactate). The blood
pressure was normal (Table 1).

DISCUSSION

In our case hypergammaglobulinemia and a posi-
tive Waaler Rose test were demonstrated when the
patient was 27. By the age of 34 he had nephro-
calcinosis and 3 years later he had frank renal
tubular acidosis. No evidence of rheumatic dis-
ease of liver cirrhosis or of Sjögren's syndrome
could be demonstrated. There were no general
manifestations suggesting SLE. Neither were there
any manifestations of purpura. The most striking

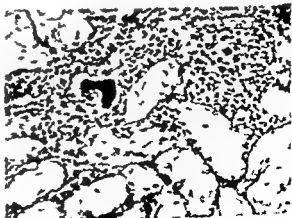


Fig. 2 Detail of Fig. 1 Dense infiltrates consisting of plasma cells. H.E., $\times 340$.

Table I *Chemical findings and body weight 1955-1968*

Therapy started Feb 1967

	1955	1957	1964	1967		1968 Dec
				Feb	April	
/ glob g/100 ml	1.9	2.1	1.7	1.3	1.9	2.1
Hb g/100 ml	12.8	12.5	11.0	9.6	9.6	11.8
NPN mg/100 ml	30	27				
Creat. mg/100 ml			1.3	1.8	1.1	1.2
K mEq/l			3.1	1.8	4.2	4.1
Na mEq/l				138	144	145
Cl mEq/l				119	112	111
Bicarb mEq/l			18	10	20	19
Ca mEq/l			4.5	4.0	4.6	4.5
Alk phosph. units			25	8	15	7
Body weight kg	72	69	66	52	69	69

finding was the plasma cell infiltrates in the kidney

Renal tubular acidosis appears as a tubular syndrome of varying origin. It may often be due to a genetic defect. Occasionally it may perhaps be ascribed to a toxic injury such as when it occurs after administration of tetracycline that has been stored too long (15). It is more difficult to explain the cases that develop in association with gammopathy. The possibility of the increased amount of gamma globulin injuring the tubules either directly or via angitis has been considered. In our case it would appear that the condition was due to tissue injury of the type seen in plasma cell hepatitis, chronic thyroiditis and Sjogren's syndrome. The actual clinical picture of renal tubular acidosis could then be conceived as a manifestation of renal injury of varying etiology. It appears natural to compare it to myxedema for example as an expression of different kinds of injury of the thyroid or of hepatic insufficiency with its varying etiology. The frequency of this type of mechanism in renal tubular acidosis is not known. But it may be suspected in all cases of gammopathy. Plasma cell infiltrates in the kidney can only be demonstrated after surgical biopsy, fine needle biopsy not giving sufficient information on the radial deposition of plasma cells. No cases with such infiltrates have been published possibly because of insufficient material available for examination. Infiltrates of plasma cells and lymphocytes have been demonstrated at autopsy of one case of Sjogren's syndrome (2). But the report of that case said nothing about

the acidifying capacity of the kidney. Bucher and Reid (2) also reported the autopsy findings in ten other cases in some of which the histological picture resembled that in our case but no mention was made of a correlation between tubular function and the histological finding. In a series of nine cases with Sjogren's syndrome kidney biopsies were studied in eight and one post mortem (13). Six of these patients had changes indicating interstitial nephritis and in one of them it was stated that latent renal tubular acidosis was present. Another series (12) with 12 patients of the same syndrome contained one patient with glomerulonephritis and four renal tubular acidosis. Five cases were biopsied and interstitial chronic inflammation was found in several preparations with numerous mature lymphocytes.

ACKNOWLEDGEMENT

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IDIOPATHIC PULMONARY HYPERTENSION IN TWO SIBLINGS

Clinical Microangiographic and Histologic Observations

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Abstract The clinical course and patho-physiologic features of idiopathic pulmonary hypertension in two female siblings aged 16 and 22 years are reported. The pulmonary vascular lesions were studied in serial histologic sections and a morphometric method was used for the determination of medial hypertrophy in the muscular pulmonary arteries. Microangiographic examination of the pulmonary arterial system was also done in one case. The pulmonary vascular lesions were similar in both cases, with prominent medial hypertrophy in muscular pulmonary arteries, as well as necrotizing arteritis, cellular intimal proliferation with plexiform lesions, and segmental fibrous obliteration. The alveolar capillaries were normal or in places dilated, and there was microangiographic and histologic evidence of collateral arterial supply of the alveolar capillary bed peripheral to obliterated segments of the pulmonary artery.

This report includes the first microangiographic description of the plexiform lesions, which could be identified as tortuous vascular channels some of which did not allow the further transmission of contrast medium.

Idiopathic pulmonary hypertension (IPH) is a comparatively rare condition particularly affecting females and usually fatal within a few years after the initial symptoms. The familial occurrence of this disorder has been reported in several communications (5 14 26). Histologically the pulmonary vascular lesions of IPH include medial hypertrophy cellular intimal proliferation intimal fibrosis, plexiform and dilatation lesions and necrotizing arteritis (31). These features are generally considered to be identical with those of pulmonary hypertension secondary to congenital heart disease. Some authors however have claimed that developmental defects in the muscular pulmonary arteries form the underlying cause of the progressive vascular changes in IPH (6 8).

Angiographic studies of the pulmonary arterial

system in IPH have revealed a pruned appearance of the peripheral pulmonary arteries (6). The fine intralobular arterial pattern however cannot be readily demonstrated by conventional angiographic techniques.

The purpose of the present paper is to describe the clinical course and patho-physiologic features of IPH in two siblings and to report the results of combined microangiographic and histologic studies of the intralobular arterial pattern in one of these cases.

CASE REPORTS

Case 1

A Latvian woman, first admitted to the hospital at the age of 25. Two years earlier a systolic murmur had been heard over the pulmonary area at a routine examination. Because she felt asymptomatic she deferred further examination at that time. She now complained of exertional dyspnea and easy fatigability gradually worsening over the last eight-ten months. The patient also complained of syncopal attacks on exertion and of dizziness on standing up. She gave a history of moderate edema of her feet during the last few weeks. There was no history of recurrent attacks of cough or fever and there was no past history of joint pains.

An elder sister (case 2) had died two years earlier of primary pulmonary hypertension. No other case of either pulmonary hypertension or other remarkable cardiovascular or congenital diseases were known in her family.

On examination at rest she showed no dyspnea or peripheral cyanosis. The jugular veins were not distended, and there was no hepatic enlargement. The second heart sound at the pulmonary area was split, with accentuation of the pulmonary component. There was a loud pulmonary ejection murmur but no diastolic murmur. A marked right ventricular heave was present. There was no radiological evidence for cardiomegaly but there was a huge dilatation of the pulmonary conus with markedly oligemic peripheral lung fields. Electrocardiogram had

Table II Data obtained with heart catheterization during rest and exercise in case 2 (supine position) 10 months ante mortem

	O ₂ uptake (ml min STPD)	O ₂ -capacity (vol %)	O ₂ sat ()		a-v O ₂ diff (vol %)
			Pulm. art	Aorta	
Rest	249	18.0	72	99	5.5
0.05 km/min	648	18.1	62	91	12.3

to this patient as in her sister but hepatomegaly ascites and peripheral edema finally became even more prominent. There were syncopal attacks but not as frequently as in case 1. The patient died in right ventricular failure at the age of 27.

The main autopsy findings were confined to the heart and the pulmonary arterial system. As in the former case there was prominent hypertrophy and dilatation of the right ventricle and atrium, but no evidence of cardiovascular malformation. The weight of the heart was not recorded. The pulmonary trunk and the main branches of the pulmonary artery were dilated and the site of wide-spread atheromatous lesions. In the right upper lobar branch of the pulmonary artery there was a yellowish-grey thrombus, firmly adherent to the vascular wall, probably an organized embolus. The lesions of the peripheral pulmonary arteries will be described separately below. The pulmonary parenchyma proper was dry brownish, without infarcts or inflammatory changes.

There was a small peptic ulcer in the stomach, at the lesser curvature close to the pylorus. The remaining organs were congested but otherwise unremarkable.

teries was measured according to the principles suggested by Wagenvoort (30, 31). This morphometric method yields a mean ratio between the medial thickness and the external diameter of the arteries.

RESULTS

Angiographic and microangiographic findings

In survey angiograms of the whole lungs of case 1 the peripheral pulmonary arteries were tortuous and there was poor capillary filling of the pulmonary parenchyma. The microangiographic pattern was essentially the same in all lung lobes with prominent irregular narrowing or total obstruction of numerous intralobular arteries (Fig 1). A few plexiform lesions were visualized in the microangiograms and tortuous vascular channels (Fig 2) some of which did not allow further transmission of the contrast medium.

MICROANGIOGRAPHIC AND HISTOLOGIC STUDIES

Methods

The lungs of case 1 were injected for 30 min with 75% aqueous suspension of fine barium sulfate (Micropaque®; Daman & Co). The injection pressure was recorded and kept at 80 mm Hg. The filling of the lungs was checked in survey radiograms. After fixation in neutral formalin, frontal slices (2-3 mm thick) of the lungs were radiographed and representative specimens from all lobes were selected for microangiography. The selected specimens were embedded in a mixture of histowax and beeswax and cut in slices 1000 μ thick, which were stereomicroangiographed by a technique described elsewhere (24). Abnormal features found in the microangiograms were correlated to the histologic appearance of the same vessels in serial sections. Areas chosen for serial sectioning were cut out of the larger blocks and re-embedded in paraffin. The histologic sections were stained with hematoxylin and eosin or with Weigert's elastic tissue stain combined with van Gieson's stain.

From case 2 only two paraffin-embedded blocks of lung tissue were available. These were studied in serial histologic sections stained as in case 1.

The medial thickness of the muscular pulmonary ar-



Fig 1 Microangiogram (case 1) showing prominent, irregular narrowing of the intralobular arteries and poor capillary filling of the pulmonary parenchyma $\times 9$

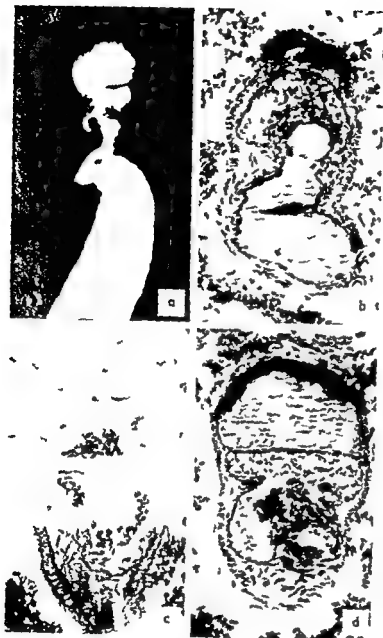


Fig 2 Plexiform lesion demonstrated in microangiogram and serial sections. (a) Microangiogram $\times 64$ The plexiform lesion stands out as a tortuous appendix to a muscular pulmonary artery (b-d) Selected serial histologic sections from upper area in (a) showing various levels of the plexiform lesion. There is some transmission of contrast through the plexiform lesion to a dilated small muscular pulmonary artery (c and d upper) $\times 162$. (b and d), Hematoxylin-eosin (c) Elastin-van Gieson.

Patchy filling of the alveolar capillary bed was demonstrated in the microangiograms even in some areas cut off from their usual arterial supply. Overdistensions of capillaries and precapillaries in the neighborhood of such areas possibly related to collateral circulation (see below) caused an irregular bushy appearance of the microangiographic pattern (Fig 3).

The contrast medium had reached the bronchial arterial system in many places probably via arterial bronchopulmonary anastomoses. Three such anastomoses were found in the microangiograms and later verified by serial sectioning. Two of

the anastomoses were pleural and of the end-to-end type. One of these (lumen diameter 320μ) was located at the mediastinal aspect of the left upper lobe and the other (diameter 200μ) at the lower interlobar fissure of the right lung. The third anastomosis (diameter 100μ) was intra-pulmonary of the end-to-side type and situated in the right middle lobe.

Histologic and morphometric findings

The histologic appearance of the pulmonary vascular lesions were essentially similar in case I and case II. There was considerable medial hyper-



Fig 3 Intralobular collateral arterial supply of the alveolar capillary bed peripheral to an obliterated segment of the pulmonary artery. The origin of the obliterated branch is indicated by small black arrow in (a) and the same artery by small black twin arrows in (b) and (c). Peripheral to the obliterated segment, the artery is patent and filled with contrast (b right upper). By means of arteriolar branches (c) corresponding arrows in a and b) the artery communicates with the precapillaries and the alveolar capillary network (b

center). The latter is also supplied by intralobular capillary precapillary communications, which give the "bushy" appearance of the microangiogram in the right upper part of (a). The extended bronchiolar precapillaries in (a) left center and (c) left center probably also represent intralobular collateral communications, though unrelated to the arterial supply of the capillary bed visible in this particular field. (a) Microangiogram, $\times 27$ (b and c) Selected serial histological sections from central area in (a) Elastin-van Gieson $\times 69$.

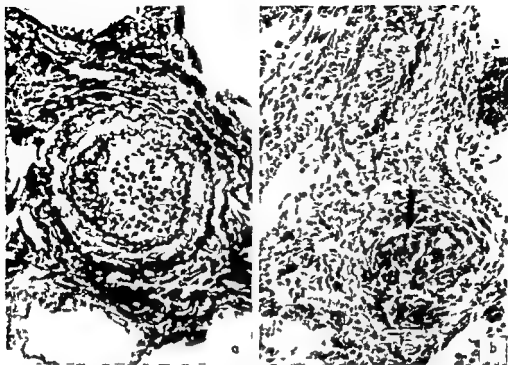


Fig 4 Pulmonary vascular lesions case 1 (a) Medial hypertrophy in muscular pulmonary artery with complete fibrous obliteration. Note the presence of lymphocytes in the fibrous tissue obliterating the lumen. Elastin-van Gieson $\times 250$ (b) Muscular pulmonary artery running from top and dividing in the center of the field. Intimal fibrosis and medial necrosis in the parent branch. The

left lower branch is completely obliterated by loose fibrous tissue. There is some perivascular lymphocytic infiltration and some deposition of anthracotic pigment. In the right lower corner there is a plexiform lesion with focal fibrinoid necrosis (arrow). Hematoxylin-eosin $\times 182$.

ophy in the majority of the muscular pulmonary arteries (Fig 4a). The morphometric studies showed a medial thickness/external diameter index of 10% (normal $\leq 5\%$) in case 1. The corresponding index in case 2 was 13%.

Cellular intimal proliferation with multiple plexiform lesions was a prominent feature in both cases (Figs 2, 4b and 5b). Focal fibrinoid necrosis was observed in the media of muscular pulmonary arteries (Fig 5a) in the cellular intimal proliferations and in the plexiform lesions (Figs 4b and 5b).

There was prominent irregular intimal fibrosis, largely concentric in type, in muscular and transitional (elastic muscular) pulmonary arteries with complete obliteration of numerous intralobular branches (Figs 4 and 5a). This process of obliteration was frequently accompanied by a vascular and perivascular lymphocytic infiltration. In many places there was deposition of anthracotic pigment

around obliterated pulmonary artery branches (Fig 4b).

The segmental character of these obliterating lesions should be stressed. Distal to an arterial segment with fibrous obliteration or a plexiform lesion, the arterioles were dilated and thin-walled. These dilated vein-like pulmonary arteries emptied into precapillaries and alveolar capillaries without obliterative features. In fact, the alveolar capillary bed appeared overdilated in some areas, possibly in relation to intralobular collateral circulation.

In places, contrast medium had reached the dilated arteriole and the alveolar capillary bed distal to an obliterated segment of the pulmonary artery. This could be demonstrated in microangiograms as well as in the serial sections, and the following alternative collateral pathways to the alveolar capillary bed were recognized:

(a) pleural and septal arteries (some of which

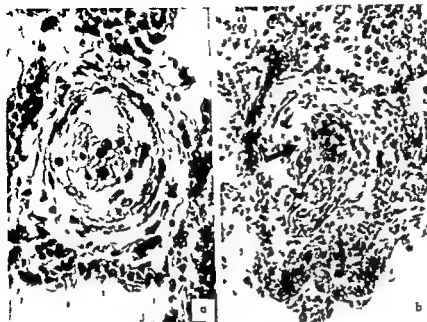


Fig 5 Pulmonary vascular lesions case 2 (a) Small, but thick walled muscular pulmonary artery with obliterating mural necrosis. Slight perivascular lymphocytic infiltration. Hematoxylin-eosin $\times 430$ (b) Plexiform lesion with focal fibrinoid necrosis (arrow). There is moderate perivascular lymphocytic infiltration. Hematoxylin-eosin $\times 170$

possibly systemic) entering the pulmonary parenchyma to ramify as alveolar capillaries

(b) capillary communications with adjacent areas supplied by patent pulmonary artery branches and

(c) overdistended bronchiolar precapillary vessels

An area with collateral arterial supply of the alveolar capillary bed is shown in Fig 3

The bronchial arteries and the pleural systemic arteries showed normal histologic features for the age with a varying amount of longitudinal intimal smooth muscle bundles leading to complete obliteration of some branches. This type of mural structure was also present in the two above mentioned arterial bronchopulmonary anastomoses of end to end type. The intrapulmonary end to side anastomosis, however, showed no obliterative features. The histologic appearance of the pulmonary and bronchial venous systems was normal.

COMMENT

The preponderance of IPH in young females, its familial incidence and its rapidly fatal course frequently with a duration of life from onset of symptoms of only 2 years or less (14) are all well known features of IPH and apply to the present two cases.

Clinically the diagnosis IPH must be established by exclusion of any apparent causative lesion e.g. left to-right shunt, intrinsic pulmonary disease or obstruction distal to the lungs. In the present two cases neither cardiac catheterization or autopsy revealed such underlying mechanisms. On the other hand the structural changes observed at autopsy probably played a significant role in maintaining the hypertension in the later stages of the disease as pointed out by Coleman et al (5).

The occurrence of dizziness and syncope after exertion is a common finding in IPH (28). Howarth and Lowe (15) have presented evidence that this may be due to acute failure of the right heart with a critical reduction in cardiac output. It is evident from Table I that the exercise cardiac output in case 2 was considerably lower than normal. When exercise is completed under these conditions and the leg muscle pump no longer acts as a booster to the cardiac pump (17) the blood supply to the brain may become inadequate due partly to persisting vascular dilatation and blood pooling in the working muscles. That the arterial oxygen saturation often shows a reduction with exercise in IPH (28) as also observed in case 2 (Table I) probably contributes to an insufficient oxygen transport to the brain.

The low arterial oxygen saturation observed

during exercise in case 2 can be attributed to the significantly reduced oxygen saturation of the mixed venous blood (cf Table II) in combination with a direct right-to-left shunt flow through precapillary systemic pulmonary anastomoses. Supporting evidence for the occurrence of significant true anatomical shunts in IPH has been obtained from the pure O_2 technique by Goff and Gaensler (9). In the present study some right-to-left flow may well have occurred through the arterial bronchopulmonary anastomoses demonstrated at the histologic examination in case 1 (see Results).

The finding that in case 1 the infusion of a vasodilator drug reduced the pulmonary vascular resistance considerably ten months before her death and only slightly four months later agrees well with earlier reports on the effects of vasodilator drugs in IPH (for review see (19)). These indicate that vasoconstriction plays a subsidiary role in the pressure elevation in the earlier stages of the disease, but not later on, when organic changes become more extensive.

The presence of probably irreversible obliterative lesions in the pulmonary arterial system was confirmed in both cases by angiographic and histologic techniques. These vascular lesions are similar to those in previously reported cases of (31). Survey post mortem angiograms from case 1 had the characteristic "pruned" appearance resembling "the denuded shrub in winter" (6) and wide spread narrowing and obliteration of the intralobular arterial bed was obvious in the microangiograms.

Peripheral to the obliterated segments of the pulmonary arteries there was a normal or in places, dilated alveolar capillary bed. This fits in with the observation by McCredie (18) that the pulmonary capillary blood volume as estimated by the D_1CO technique may be maintained in IPH. Our study also confirms the suggestions of some previous investigators (11, 23) that, in the presence of hypertensive obliterating arterial lesions, the intact alveolar capillary bed is to some extent supplied by intrapulmonary and pleural collateral pathways.

The patent arterial bronchopulmonary anastomoses that were demonstrated by microangiography and serial sectioning in case 1 further reflect the increased collateral arterial supply of the pulmonary parenchyma. Such anastomoses

are normally present in the fetal and infant lung (24, 33) but not in the adult lung (27). The anastomoses in case 1 however seem to be few in number and our study fails to support the theory of abnormal arterial bronchopulmonary anastomoses as the primary cause of IPH (2, 4, 7, 34).

The possible role of occult pulmonary thromboembolism in the pathogenesis of IPH has been considered by some authors (3, 12, 16). In one of our cases an organized embolus was found in a lobar branch of the pulmonary artery. However single organized pulmonary emboli of this size do not seem to be able to initiate persistent pulmonary hypertension. Furthermore since the mural changes in the peripheral pulmonary arteries of our two cases suggest gradual cellular intimal proliferation with eventual fibrous obliteration, we do not believe that chronic pulmonary thromboembolism was the cause of IPH in these two siblings.

The present report includes the first angiographic documentation of the plexiform lesions, a highly characteristic feature of severe pulmonary hypertension. Previous studies on the structure of the plexiform lesions with serial sectioning have shown that this lesion is entirely arterial and that its efferent vessels terminate as alveolar capillaries (1, 21, 22, 29). Our microangiographic findings confirm these earlier observations. Some of the plexiform lesions demonstrated in case 1 allowed the transmission of contrast into dilated arterioles (Fig. 2) which in the serial sections could be traced down to alveolar precapillaries and capillaries.

Although vasoconstriction may have contributed to the increased medial thickness of the muscular pulmonary arteries, our morphometric results strongly suggest true medial hypertrophy. This is particularly true for case 2 in which the pulmonary arterial system was injected and hence to some extent distended by contrast medium. The medial index of this case (10%) was nevertheless twice the normal. Medial index values above 10% are ordinarily not encountered after the age of one month (32). It cannot be determined from our study, however, whether the medial hypertrophy is secondary to the hypertensive state or whether there is some primary carry-over of the fetal medial structure in the muscular pulmonary arteries as has been suggested in cases of IPH occurring in infancy (10, 13, 20, 25).

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Congress Announcements

The Third International Congress of Social and Preventive Medicine will be held in Lido of Venice Italy May 24 to 28 1970

Information Segreteria del III° Congresso Internazionale di Medicina Preventiva e Sociale Ospedale al Mare 30126 Lido di Venezia Italy

The Second Czechoslovak Congress of Internal Medicine with International Participation on the Topic of Pathogenesis and Therapy in Edema will be held in Bratislava September 15 to 18 1970

Information Congress Office First Clinic for Internal Diseases Mickiewiczova 13 Bratislava Czechoslovakia

The Second Congress of the European Association of Radiology will be held in the International Congress Centre RAI in Amsterdam June 14 to 18 1971

Information The Congress Secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague Holland

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